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and

Steroid Hormone Administration

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CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY

VOLUME III

Hormones, Psychology and Behaviour
and
Steroid Hormone Administration

General Editor for the Ciba Foundation

G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., B.Ch.

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With 78 Illustrations



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PREFACE

THE Ciba Foundation is an international centre where workers active in medical and chemical research are encouraged to meet informally to exchange ideas and information. In two and a half years since its opening in June, 1949, in addition to many part-day discussions, there have been 13 international symposia, each lasting two to four days, attended on invitation by outstanding workers from many countries.

The informality and intimacy of these meetings have permitted discussion of current and incomplete research and stimulated lively speculation and argument. They have also been the occasion for reference to much published and unpublished work throughout the world. The proceedings are now being issued in full, with only the minimum of editing, in order to pass on to a far wider audience the benefits of these meetings. Assembled in book form they present very readably much information not readily available elsewhere.

Nine of the first 13 Symposia form a series of "Colloquia on Endocrinology," dealing mainly with steroid hormone problems. One of these, on Nomenclature of Steroids, has had its conclusions published separately,* of the remaining eight, two are now combined in each of four volumes.

This third volume contains in its first part the papers and full discussions of a conference held originally under the title of The Influence of Steroid Hormones on Psychological and Behavioural Reactions. The participants came from unusually diverse disciplines, and included biochemists, experimental physiologists, zoologists, clinicians and psychiatrists. Much new ground, not previously of common knowledge and interest to all members of this group, was covered during

*Chemistry and Industry, June 23rd, 1951.

consideration of psychological and behavioural reactions with normal and with pathological secretion of hormones and following hormone administration. The discussion ended with a debate, reported in full, on "methodology".

Book II of this volume contains the proceedings of the first of the Ciba Foundation colloquia on endocrinological subjects, on Administration and Dosage of Steroid Hormones. No manuscripts were demanded or prepared on that occasion, and the informal contributions, both of programme speakers and speakers in the discussions, have been reproduced from recordings. Information was pooled on such matters as the use of injections, emulsions, nasal sprays, and particularly subcutaneous pellets of steroid hormones in man and animals in relation to the effects produced, the bioassay of progesterone and pharmacological assay of synthetic œstrogens, and excretion studies after hormone administration by different routes.

The facts from much experimental work and the speculative nature and frankness of the discussions, with easy reference to important trivialities not normally mentioned on more formal occasions, should make volume III both stimulating and agreeable reading.

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G. PINCUS	Worcester Foundation for Experimental Biology, Shrewsbury, Mass
M REISS	Bristol Mental Hospitals
C. P. RICHTER	Johns Hopkins Hospital, Baltimore
S. L. SIMPSON	St. Mary's Hospital, London
A. W. SPENCE	St. Bartholomew's Hospital, London
A. WALTON	A.R.C. Unit of Animal Reproduction, Cambridge
S. ZUCKERMAN	University of Birmingham

List of those participating in or attending the Conference on the Influence of Steroid Hormones on Psychological and Behavioural Reactions, 9th-12th April, 1951.

C. E. ALLEN . .	Dreadnought Seaman's Hospital, Greenwich
E C. AMOROSO . .	Royal Veterinary College, London
F. A. BEACH	Yale University
L. R. BROSTER .	Charing Cross Hospital, London
J S L. BROWNE .	Royal Victoria Hospital, Montreal
R. A. CLEGHORN .	Allen Memorial Institute of Psychiatry, Montreal
A. T. COWIE .	National Institute for Research in Dairying, Reading
E. W. DEMPSEY . . .	Washington University, St. Louis
J. T. EAYRS . . .	University of Birmingham.
F. A. ELLIOTT	Charing Cross Hospital, London
S. J. FOLLEY .	National Institute for Research in Dairying, Reading
G. L. FOSS	Bristol
R. GREENE .	Royal Northern Hospital, London
J HAMMOND, SR. .	*University of Cambridge*
J. L. HANCOCK .	A.R C. Unit of Animal Reproduction, *Cambridge*
G. W. HARRIS . .	University of Cambridge
H. HEDIGER . .	Zoologischer Garten, Basel
H. KALMUS . .	University College, London
M. KLEIN . . .	Université de Strasbourg
A. J. LEWIS . . .	*Institute of Psychiatry, London*
L. H. MATTHEWS . .	Zoological Society of London
P. MESCHAKS . .	*Royal Veterinary College, Stockholm.*
A. S. PARKES . . .	National Institute for Medical Research, London

BOOK I

HORMONES, PSYCHOLOGY AND BEHAVIOUR

List of those participating in or attending the Conference on Steroid Hormone Administration, 23rd to 24th February, 1950

P. M. F. Bishop	Guy's Hospital, London
J. A. Broom	Boots Pure Drug Co., West Bridgford, Notts
G. Brownlee	King's College, London
A. T Cowie	National Institute for Research in Dairying, Reading
R. Deanesly	National Institute for Medical Research, London
E. C. Dodds	Courtauld Institute of Biochemistry, Middlesex Hospital, London
S. J. Folley	National Institute for Research in Dairying, Reading
T. R. Forbes	Yale University
G. L. Foss	Bristol
J. H. Gaddum	University of Edinburgh
F. Gross	Ciba Ltd., Basle
C. Hamburger	Statens Seruminstitutet, Copenhagen
J. Heer	Ciba Ltd., Basle
M. Klein	Université de Strasbourg
M H. I. Macaulay (now Mrs. M. H. I. Dodd)	Gatty Marine Laboratory, St. Andrews
F. H Malpress	Queen's University, Belfast
G. A. Overbeek	N. V. Organon, Oss, Holland.
A. S. Parkes	National Institute for Medical Research, London
A. W. Spence	St. Bartholomew's Hospital, London
A. Westman	Caroline Hospital, Stockholm

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CHAIRMAN'S OPENING REMARKS

S. ZUCKERMAN

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present state of the subject which it is our business to discuss. I think that gatherings of this kind are probably the best scientific meetings that can be organized. In the United States their [illegible] Laurentian [illegible] big a part. [illegible] by the Cent[illegible] reason why all these meetings are so successful is that they are small; that those present are experts, and that all who attend can bring something to bear upon a common problem. I know that those of you who have taken part before, and those of you who are taking part for the first time in a Ciba Foundation Conference will share my view about their usefulness. The first thing that I would like to do, therefore, is to express thanks to the Foundation and its Trustees for having organized this meeting.

The subject which we are to discuss, "the influence of steroid hormones on psychological and behavioural reactions," is an extraordinarily wide one. The papers that will be given during the next four days range from experimental enquiries into the excretion of neutral steroids in the urine of fertile and infertile bulls, just to quote the full title of one communication, to the influence of various hormonal factors on psychoses. The field is so wide that I doubt very much whether any single individual in this room—bar possibly two whom I wish to single out by name, Dr. Curt Richter and Dr. Beach, both of whom have ranged over the whole subject—is

CHAIRMAN'S OPENING REMARKS

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I AM deeply conscious of the honour and privilege of being Chairman of this Colloquium. It brings together a number of people, many of us friends of long standing, from the four corners of the globe, and has the function of combining diverse views and different disciplines, to provide a picture of the present state of the subject which it is our business to discuss. I think that gatherings of this kind are probably the best scientific meetings that can be organized. In the United States their counterpart in the field of endocrinology are the [illegible] reason why all these meetings are so successful is that they are small; that those present are experts; and that all who attend can bring something to bear upon a common problem. I know that those of you who have taken part before, and those of you who are taking part for the first time in a Ciba Foundation Conference will share my view about their usefulness. The first thing that I would like to do, therefore, is to express thanks to the Foundation and its Trustees for having organized this meeting.

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a specialist in more than one particular aspect. What we have to do, therefore, is to fit together our particular contributions to make a pattern of knowledge that covers the whole field.

The idea that differences in behaviour may be conditioned by factors we now recognize as hormonal, reaches back into man's distant past, in so far as the practice of castration has an immensely long history, and since man has presumably long been aware that the behaviour of the intact male differs from that of the castrate. Hormones as such were put on the map by Berthold little more than 100 years ago. It is some time since I read his paper, but I seem to remember that in the two to three pages it comprises, he refers to crowing and masculine behaviour as being induced in capons into which he had implanted testicular fragments. The experiments of Brown and Séquard in France in the latter part of the 19th century again laid great emphasis on changes in behaviour as being due to what we recognize today as endocrine action. The enormous extent to which behaviour is so conditioned became increasingly clear after the formulation of the concept of hormones in 1905 by Bayliss and Starling. Here we should pay tribute to one of [illegible] this century, W. B. [illegible] "Bodily Changes in Pain, Hunger, Fear and Rage," underlined the fact that many common features in the behaviour of vertebrates are mediated by hormonal action. Many a popular book has since been based on the view that differences in personality are due to different endocrine factors, and some authors have also thought that evolutionary change can be explained as due to changes in endocrine system. Thus, in a book produced just before the war, Marriott attributed all the racial differentiations of humanity, morphological and [illegible]

[illegible] perspective [illegible] eat pleasure

PART I

PSYCHOLOGICAL AND BEHAVIOURAL REACTIONS CONNECTED WITH THE PHYSIOLOGICAL PRODUCTION OF STEROID HORMONES

SEX AND SPECIES DIFFERENCES IN THE BEHAVIOURAL EFFECTS OF GONADAL HORMONES*

FRANK A. BEACH

THERE are several differences in the effects of gonadal hormones upon the behaviour of males and females within various mammalian species, and there are other differences between species. Some of these differences seem related to variations in the functions of the central nervous system. For this reason I shall summarize briefly a number of experiments dealing with the neural basis for reproductive behaviour.

Brain Functions in Mating Behaviour

Goltz (1874) reported that female dogs deprived of both cerebral hemispheres are capable of fertile coition, and the more systematic studies of Bard (1936) indicate that the same is true of female cats. Dempsey and Rioch's findings (1939) show that copulatory reactions survive in decorticated female guinea pigs. Detailed measurements of coital behaviour in female rats before and after extensive neocortical injury have also been published (Beach, 1943, 1944).

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*All of the author's researches mentioned in this article have been supported by the Committee for Research in Problems of Sex, National Research Council.

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Experiments upon male animals point to a different conclusion. After removal of various amounts of neocortex male rats become sexually less active. As demonstrated in figure 2, lesions involving less than 20 per cent of the total

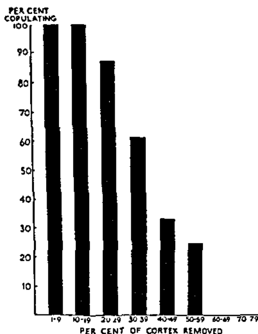


FIG. 2. Effects of partial decortication on mating in male rats. Per cent of males in each lesion group continuing to copulate after operation. (Beach, 1940.)

cortical mass have no effect upon the proportion of animals showing copulatory reactions. But loss of more than one-fifth of the cortex results in a lowering of this particular measure of sexual activity. The reduction is progressive as

One index to sexual receptivity in this species is the ease with which a female can be induced to display the lordosis response which is essential to the male's achievement of intromission. The *copulatory quotient* is a score based upon the number of times the female is mounted by the male and the number of times she responds with lordosis. This measure was unaffected by unilateral hemidecortication and by [illegible] There was [illegible]

(" normal ") scores, tended to persist after brain injury.

Figure 1 reveals that in these same animals there occurred a postoperative increase in the frequency of such œstral

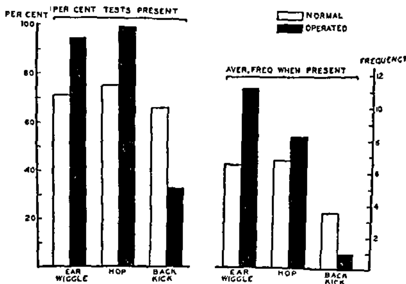


FIG. 1. Effects of removing the cerebral cortex on sexual behaviour in female rats. (Beach, 1943.)

reactions as wiggling of the ears and hopping. In contrast, back-kicking, which is a method of repulsing the male, occurred less frequently in decorticated than in normal females. On the basis of these and other data presented in

the original reports it can be concluded that an intact cerebral cortex is not essential to sexual excitability and mating behaviour in the female rat. The evidence pertaining to dogs, cats, guinea pigs and rats suggests that in these species also many of the basic feminine sexual reactions can be mediated by non-cortical neural mechanisms.

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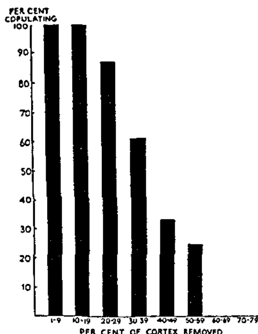


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cortical mass have no effect upon the proportion of animals showing copulatory reactions. But loss of more than one-fifth of the cortex results in a lowering of this particular measure of sexual activity. The reduction is progressive as

lesion size increases, and in the experiment here mentioned (Beach, 1940) mating was not shown by any rat if more than 60 per cent of the cortex had been removed. It is important to note that the precise locus of injury was relatively unimportant in producing this loss of sexual performance. The amount of cortex destroyed was the critical factor.

When cortically operated male rats copulate, they do so in approximately normal fashion. Their behaviour reveals no serious interference with essential sensory or motor elements involved in mating. The effect of cerebral loss appears to be a general lowering of responsiveness to the receptive female. This is brought out indirectly by the data summarized in figure 8. Here are included only the scores relating to performance of those males that continued to copulate after

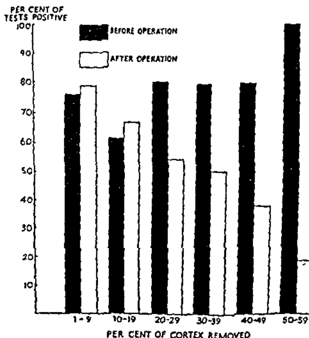


FIG. 8. Effects of partial decortication on mating in male rats. Average per cent of tests in which copulation occurred before and after operation for the postoperative copulators in each lesion group. (Beach, 1940.)

cortical injury. Nevertheless the effect of increasing lesion size is still apparent. The proportion of tests in which coital behaviour appeared was inversely related to lesion magnitude.

In this experiment rats that failed to copulate after brain operation were injected with testosterone propionate and some animals with relatively small lesions displayed a return of active sexual behaviour. This finding, in conjunction with other data reviewed above, suggests that the cerebral cortex acts as an excitatory mechanism which elevates levels of activity within lower neural centres specifically concerned with the mediation of copulatory behaviour. When large amounts of cortical tissue are removed there is a reduction in responsiveness of the lower centres to exteroceptive stimulation and sexual behaviour is unlikely to occur. These same executive centres may also be stimulated by androgen, and under appropriate circumstances the loss of excitability occasioned by restricted cortical invasion may be compensated by artificial elevation of the normal androgen level.

According to the hypothesis presented here the rôle of the cerebral cortex in masculine and feminine sexual activities is not the same, and confirmatory evidence is available in studies of male and female coital reactions shown by the same individual animals (Beach, 1943) Many female rats exhibit male-like copulatory responses when placed with a second female that is in œstrus. The most common masculine responses are mounting and clasping the other female. More intense reactions are the palpating of the stimulus animal and execution of pelvic thrusts. Females showing this behaviour are in no way abnormal. When they are in œstrus they respond in receptive fashion to the approach of [illegible]

total decortication completely eliminates the tendency to mount and clasp a second female. Thus, within the same individual, the masculine and feminine patterns of sexual performance are separately mediated, and by decortication one can be eliminated without materially affecting the other.

lesion size increases, and in the experiment here mentioned (Beach, 1940) mating was not shown by any rat if more than 60 per cent of the cortex had been removed. It is important to note that the precise locus of injury was relatively unimportant in producing this loss of sexual performance. The amount of cortex destroyed was the critical factor.

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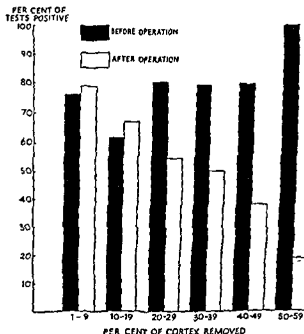


FIG. 8. Effects of partial decortication on mating in male rats. Average per cent of tests in which copulation occurred before and after operation for the postoperative copulators in each lesion group. (Beach, 1940.)

frontal lobe almost always abolished copulation. Animals operated in this fashion demonstrated strong and persistent excitement. They followed the female continuously throughout the 60-minute mating tests and repeatedly attempted to effect sexual union. But the marked sensory-motor disabilities consequent to the brain injury prevented completion of the essential bodily adjustments. Male cats lacking all of the neocortex never followed or attempted to mate with the œstrous female. Their behaviour was comparable to that of male rats with similar brain lesions.

Two points deserve emphasis. First, in cats as in rats, the male's coital performance depends heavily upon the cortex of the brain and cannot be carried out in the absence of this part of the central nervous system. But the female is capable of fertile copulation after total loss of the same brain structure. Second, unlike the male rat, the partially decorticated cat may evince marked sexual arousal even though he is unable to carry out the necessary reactions involved in coition. This latter point undoubtedly reflects the increased corticalization of sensory and motor functions in which the carnivore differs from the rodent.

There are no data to reveal the rôle of the cortex in the sexual activities of male and female primates, but it can reasonably be presumed that severe interference with basic sexual responses would follow destruction of the sensory and motor regions of the monkey or ape brain. Whether there would also occur a lowering of sexual excitability remains to be determined. It can be supposed, however, that advances in the degree to which cortical mechanisms control the performance of all types of delicate physical responses would be reflected in deterioration of the male primate's ability to copulate. The irreducible minimum of bodily adjustments necessary for fertile coition in females might remain after extensive cortical injury as long as the animal was capable of maintaining a standing posture.

The main conclusion suggested (but by no means established) by available evidence is that increasing corticalization of voluntary behaviour and of complex sensory integrations produces increasing dependence of the mating performance upon the neopallium.

Although, as mentioned earlier, female cats continue to mate after complete loss of the cortex, the same is not true in the case of males of this species. In collaboration with Dr. Arthur Zitrin, I have studied the mating performance of more than 20 male cats before and after injury to the cerebral cortex. Briefly summarized the results were as follows: Partial bilateral invasion of the occipital, temporal or parietal lobes had no effect upon copulation. Complete removal of

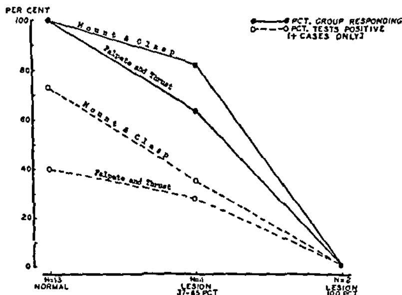


FIG. 4. Effects of cortical injury on masculine behaviour in female rats. (Beach, 1943.)

the cortex from one hemisphere produced some motor and sensory abnormalities but did not prevent successful coition. Loss of the cortex from one hemisphere combined with destruction of the contralateral occipital lobe prevented visual orientation to the receptive female; but cats with such lesions were able to copulate if they came into bodily contact with the female.

Unilateral decortication combined with loss of the opposite

It was noted above that females of several mammalian species including the rat may display masculine mating responses under certain conditions. Here it is worthwhile to add the fact that for most of these species male behaviour appears in prepuberal females and survives ablation of the ovaries in adult animals. In most cases, therefore, masculine coital reactions in the female are much less affected by ovarian hormones than are feminine responses. We have already seen that in the female rat the male pattern depends upon the cerebral cortex whereas feminine responses continue in the decorticated individual.

Longitudinal studies of sexual patterns in male animals demonstrate that male rodents, carnivores and ungulates begin to exhibit some mating reactions long before puberty. This is true of sheep, dogs, lions, guinea pigs, and rats. In the last-named species the prepuberal performance is not dependent upon testicular hormone, for it occurs with normal frequency in males castrated at birth. There is therefore the possibility that certain elements in the masculine mating pattern occur independently of the presence of testicular hormone. The appearance of masculine behaviour in immature females strengthens this hypothesis. It is essential to state that the sexual activities of prepuberal males are weak and incomplete in comparison with the performance of the adult animal. But the occasional occurrence of recognizably sexual patterns of response is in distinct contrast to the absence of feminine behaviour in females of the same species.

The sexual activities of immature monkeys and apes are more frequent and more nearly complete than are those of infra-primate males. Some chimpanzees experiment with and practice both homosexual and heterosexual patterns many years before adolescence (Bingham, 1928). I take this as a sign that the male primate's coital performance is less dependent upon testicular secretions than is the sexual behaviour of males from lower levels of the phyletic scale.

This conclusion is rendered plausible by evidence gained from studies of the behavioural effects of castration. Prepuberal castration of male rats, guinea pigs and dogs prevents the appearance of any but the most fragmentary sexual responses. Some mounting behaviour may occur during

Gonadal Hormones and Behaviour

There are several ways of studying the effects of gonadal hormones upon behaviour. One method is to observe relations between ontogenetic development of sexual reactions and developmental changes in hormone output. By this means it is possible to show that in those species of rodents and carnivores that have been carefully investigated, the initial appearance of feminine coital responses corresponds closely to the first œstrus. No female sexual reactions are seen in the behaviour of prepuberal rats, rabbits, cats or dogs. But the first time that follicles ripen in the ovaries and œstrogen secretion increases, mating reactions appear.

Immature female primates, in contrast, engage in sex play long before the ménarche. Infant monkeys and apes can be observed in partial coition with males. Their coital pattern is clear-cut and recognizable although it is not as vigorous *nor as complete as that of the adult female in œstrus.*

A second way of estimating the importance of gonadal hormones to coital performance is to study the relation between cycles of ovarian activity and cycles of sexual behaviour in adult animals. The correlation is very high in the case of rodents and carnivores. Female rats, guinea pigs, hamsters, dogs and cats rarely if ever engage in sexual relations except during the œstrus period, at which time œstrogen levels are high and mating can result in conception. Some female monkeys and apes will permit an insistent and aggressive male to copulate at times when they are not in œstrus. This does not occur frequently and the female's reactions under such conditions do not convey the impression of high sexual desire. Nevertheless the fact that some *female primates are capable of coition during the non-œstrous* period sets this group apart from the infra-primate mammals.

Evidence based upon ontogenetic development of copulatory responses and correlations between ovarian and behavioural cycles combines to reveal a slight but definite lowering of the female primate's dependence upon ovarian hormones for facilitation of her sexual responses. The extension of this tendency to the human species is obvious. Compared with females of lower species, the woman's reliance upon ovarian hormones as a source of erotic stimulation is slight.

It seems that in the case of infra-primate mammals the major elements of the female's mating pattern can be mediated by non-cortical parts of the central nervous system, although it is highly probable that in the intact animal cortical processes contribute to the integration and orientation of mating behaviour. At this same level of the evolutionary scale ovarian hormones are essential to feminine sexual behaviour, and it may be suggested that one of their principal effects is to heighten the responsiveness of the subcortical nervous mechanisms to external stimulation.

In male rodents the mediation of the motor patterns of courtship and coitus probably depends, as it does in the female, upon extra-cortical centres and circuits. But in this instance the reactivity of these centres and circuits is affected by activities occurring within the cerebral cortex. Loss of the cortex results in profound reduction of the stimulability of non-cortical mechanisms for sexual performance. Testicular hormone apparently acts directly upon the extra-cortical portions of the neural factors involved in coital activity, serving to increase the likelihood that they will respond to exteroceptive stimuli.

Female carnivores differ from males of their species in two ways. The feminine copulatory reactions are not dependent upon cortical facilitation, whereas masculine performance relies heavily upon certain cortical regions for the integration and control of the motor pattern. In addition the male carnivore may become totally unresponsive to sexual stimuli as a result of complete decortication. The second difference is reflected in the disappearance of female coital activity with the withdrawal of ovarian hormones and the survival of masculine sexual reactions in the absence of testicular secretions.

It seems possible that in both rodents and carnivores the [illegible]

be suggested that the male carnivore's continued potency in the absence of testicular hormone, as contrasted with the deterioration of this function in the castrated rodent, is related to the fact that sexual performance in the carnivore

adulthood but it is probably no more intense or frequent than similar behaviour seen in the prepuberal male. Male apes that are castrated in infancy may display vigorous coital performance when they become adult. The speed and frequency with which they copulate compares favourably with the performance of an intact animal. Only the ejaculatory reflexes are missing from their coital pattern (Clark, 1945).

The effects of castration during adulthood vary with the species and the individual. Male rodents tend to lose the ejaculatory response within a week or two after the operation. The frequency with which intromission is achieved declines progressively during the first post-operative month. An occasional act involving intromission and some mounting responses without insertion continue to occur for an indefinite period. In this survival of some slight degree of sexual ability the castrated male differs from the ovariectomized female whose receptive responses are completely and permanently eliminated.

An investigation currently in progress in my laboratory indicates that at least some carnivores react differently to castration during adulthood from male rodents. One group of male dogs has been tested for mating responses for approximately one year before and two years after gonadectomy. The average sexual performance declines progressively after removal of the testes, but nearly all animals retain some ability to penetrate the receptive bitch even two years after the operation. Furthermore there are at least a few individuals in which there occurs no detectable loss of sexual responsiveness or ability to copulate. In point of fact some dogs are more vigorous and potent two years after castration than they were preoperatively.

Conclusions and Interpretation

The evidence at hand is so fragmentary that one hesitates to suggest anything more than the most tentative sort of an interpretation. The following remarks should be regarded as a set of working hypotheses which are formulated as points of departure for more systematic and satisfactory experimental study of the basic problems at hand.

appeared under androgen, and after ejaculating, the male would be refractory to sexual stimulation for a certain period of time. There are no comparable studies on monkeys as far as I know.

ZUCKERMAN: I recall some experiments of my own—I am afraid the details escape me now—on a male drill, and on a few male rhesus monkeys, which suggested that after castration sexual activity declined rather considerably. In the case of the drill I certainly remember that its sexuality became intensified after injection of androgen. The other thing I remember is that the intact adult male chimpanzee may *occasionally* manifest weak sexuality.

RICHTER: Do you think that the œstrogen and androgen from the adrenals might play some part?

BEACH: Certainly this is a possibility worthy of investigation.

PINCUS: The secretory activity of the adrenals in species other than the monkey might be quite different. For example, in the human there is evidence of androgens produced in fair amount, whereas œstrogen is produced in relatively small amounts.

BEACH: But so far as I am aware there is no evidence that indicates any *behavioural* effect of adrenal androgens.

BROWNE: There is certainly evidence which I will produce at a later time that the injection of ACTH in panhypopituitary cases has an effect on human behaviour also from the point of view of sexuality.

BEACH: I hope to get more direct evidence on this point by adrenalectomizing some of our castrated dogs.

BROWNE: Do you not feel that as a general principle the fact that adrenalectomy or the removal of the second gland has no further influence is perhaps only part of the evidence, and that probably the aspects of, shall we say, the production of androgenic or œstrogenic effects by *stimulating the adrenals should also be examined?*

BEACH: Yes.

ZUCKERMAN: I take it, Dr. Beach, you would not suggest that an animal deprived of all its endocrine tissues other than its gonads would behave sexually, would you?

BEACH: No.

ZUCKERMAN: I think we can assume that a normal metabolic state would be necessary for these waves of sexual behaviour to be manifested.

[illegible]

[illegible]

is more completely corticalized than it is in the rodent.

Finally, in considering the subhuman primates, two generalizations may be offered. Both males and females are considerably less dependent upon gonadal hormones for sexual behaviour than are animals of infra-primate types. And there is some indication that males are more free from this type of hormonal control than are females. This may indicate that even among the primates there exists a marked difference in the degree to which the cerebral cortex controls and contributes to sexual activities.

The bearing of the foregoing generalizations upon physiological interpretations of human sexual practices is evident. It is clear that in our own species there exists the most marked and obvious emancipation of erotic responsiveness from hormonal control (Ford and Beach, 1951). Many men and women are capable of full sexual arousal and satisfaction in complete absence of the gonads and their secretions. This, I believe, is a direct consequence of the extreme dependence of human behaviour upon the complex and intricately organized cerebral cortex.

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DISCUSSION

ZUCKERMAN: Do I understand that the view is that the male chimpanzee and monkey does not lose his interest in sexual behaviour after castration?

are being artificially inseminated, and the remark has been made that the activity of the castrated males falls off very severely about three months after castration.

BEACH: This is where one *must* have quantitative tests and controlled pre-castration experience.

HAMMOND: In the boar sexual activity, rather less than normal, goes on for three to four months at any rate, but there is still considerable activity three months after castration (Wallace, 1949, *J. Endocrinol.*, 6, 205).

ALLEN: I would like to ask Dr. Beach whether the aggressive sexual behaviour, apart from the performance of the sexual act, is affected by castration. Does the castrated male fight at the rutting time like the stag? I am interested in this, because some Scandinavian countries castrate their sexual delinquents, and in sexual delinquency it must be the aggressive part which is more dangerous than the actual sexual act. And if they do get any good results, and they claim they do, but I am sceptical, then it would be because the aggressive side is reduced.

BEACH: Certainly in lower animals aggressive behaviour is reduced by castration, and administration of androgen increases it. But if, as I believe is the case, hormonal influences are less important in higher animals, androgen might have less of an effect upon aggressive behaviour in humans as well as upon primary sexual behaviour.

ZUCKERMAN There is a fair amount of evidence that human castrates may remain aggressive, is there not?

BEACH: A lot has been written about the subject but as far as I know no convincing evidence has been reported. It all seems to be anecdotal.

CLEGHORN: There is a report on the administration of stilbœstrol to sexually aggressive criminals, with alleged increase in socialization.

WALTON: I would like to ask whether any attempts were made to assay the testosterone level in these animals? The work of Parsons and Mann has shown that probably one of the most sensitive tests of testosterone is the concentration of fructose in the semen. This can be assayed by using an artificial vagina to collect the semen and estimating fructose in the ejaculate. One can then determine how rapidly removal of the testis alters the fructose level It would be very interesting, I think, if this procedure were carried out in these castration studies. Parsons and Mann did their work on the rabbit, and found that it was quite possible to maintain ejaculation in one animal for several months after castration, but after the first week there was an almost immediate loss of testosterone activity as measured by the fructose content of the semen.

BEACH: We have assayed the urine for total 17-ketosteroid content, and find no statistically reliable difference between the urine of normal dogs and others that have been castrated for 18 months There is a tremendous range of individual variation in normals, and there is no relationship among normals between the 17-ketosteroid level and the sexual behaviour. We have not analysed the semen from our animals

ZUCKERMAN: Dr. Walton, in general does a post-pubertally castrated member of the *Bovidæ* behave like a dog or a rat?

WALTON: I think there is a very considerable loss of sexual activity after castration of bulls, but you do get mounting behaviour in the castrated animal. It is difficult to get really accurate data.

HANCOCK: The only information I have on it is from other people's field experience. It is common practice in one part of England to castrate bulls and use them as teasers for intact females in œstrus which

muscular patterns of response and in the sensory stimuli adequate to release these patterns. A possible explanation of these sex differences is that stimuli arising in the differently-organised reproductive systems of male and female are selectively linked to a sequence of behaviour characteristic of the homologous sex. But since the essential pattern of mating behaviour survives the removal of the reproductive tract (Ball 1934), it is more likely that such stimuli augment rather than initiate the performance of the response. Furthermore, although gonadectomy either eliminates or considerably reduces any sex differences in behaviour, these can be restored by administering small doses of the homologous sex hormone to both male (Beach and Holz, 1946) and female (Beach, 1942*b*) notwithstanding the absence of the gonads. It is generally assumed, therefore, that sex differences in behaviour are primarily due to changes in the central nervous system wrought by the activity of the sex hormones and are not the secondary effects of differences in sensory input.

Nervous and Endocrine Factors in Motor Activity

It appears, however, that the apparatus for combining simple neuromuscular patterns of activity into the sequences characteristic of the instinctive behaviour of either sex, is bisexually represented; and that there is no truly specific relationship between any one hormone and the pattern of behaviour which it facilitates. For instance, while partial sex reversals in behaviour can be induced by treating prepubertally-castrated male (Ball, 1939) and female (Koster, 1943) rats with the sex hormones of the opposite sex, these same hormones will, if given in suitably large doses, elicit homotypical behaviour (Ball, 1937; Beach, 1942*c*). Furthermore, many components of maternal behaviour (which is generally regarded as an attribute of the female) can be [illegible]

which will restore the level of spontaneous activity in the spayed rat (Hemmingsen and Krarup, 1937). In their

SEX DIFFERENCES IN THE MATURATION AND FUNCTION OF THE NERVOUS SYSTEM IN THE RAT

J. T. EAYRS

The concept of a hierarchy in the organisation of instinctive behaviour (Weiss, 1925, 1941; Tinbergen, 1942) implies that the central nervous system may be regarded in terms of superimposed and mutually integrated levels of function, activity at higher levels determining the release of neuromuscular patterns of behaviour which are "set" in the organisation of the lower. In the rodent, no sex difference has yet been reported in the development or performance of the basic neuromuscular patterns nor of their simpler combinations, and it is in more complicated forms of behaviour, which owe their appearance to integration at suprasegmental levels, that the responses of male and female to environmental change differ both qualitatively and quantitatively. How far this is due to differences in neural organisation, and how far to differences in reactivity to the *sex hormones, has not yet been conclusively established.* Much work has, however, been done towards elucidating the problem.

Sex Differences in Adult Behaviour

In the rat, with which this communication is primarily concerned, the differences between the two sexes have been extensively studied in relation to general locomotor activity, and to the specific patterns of sex and maternal behaviour. Thus the male is less active than the female, and shows none of the rhythmic activity associated with the ovarian cycle (Wang, 1923; Slonaker, 1924; Richter, 1927); and both in prepubertal sex-play (Beach, 1942*a*) and adult mating behaviour there are wide differences both in the neuro-

Sex Differences in Neural Structure

Clearly, then, sex differences in behaviour must be interpreted in terms of the interaction between central nervous organisation and endocrine factors.

Beach (1948) suggests that both sexes possess the central nervous mechanisms necessary for performing both male and female patterns of behaviour. One of these mechanisms is dominant and has an affinity for the homologous sex hormone, although it can, with greater difficulty, be excited by that of the opposite sex. A similar view has been proposed by Ball (1939). Underlying this hypothesis is the implication of a structural difference between the male and female nervous systems which renders one more susceptible than the other to the action of specific steroids

No such difference has yet been adequately investigated, but recently Barr, Bertram and Lindsay (1950) have demonstrated the presence of a small nucleolar " satellite " in the nerve cells of the female which is not readily visible in the male. Histochemically this structure differs from the nucleolus and it is possible, therefore, that the female may possess an enzyme system, poorly developed in the male, which is concerned with the differential action of the sex hormones. Recently we began to investigate the relationship between the nucleolar satellite and the sex hormones during the early development of the rat, by studying its appearance in newborn males feminised *in utero* by the administration of œstrogen. The rat was found to be unsuitable for this study, and the results were on this account inconclusive; but the significance of the " satellite " in relation to the action of steroid hormones is a subject which should receive further attention.

Sex Hormones and the Maturation of Innate Behaviour

The administration of sex hormones to immature rats either directly (Beach, 1942*c*) or indirectly, by giving gonadotrophic hormone (Smith and Engle, 1927; Cole, 1936), advances not only the development of the reproductive organs but also the appearance of mating behaviour. Since the more

relationship with the nervous system, therefore, the sex hormones may be placed in a class of substances which *increase the ability of environmental stimuli to evoke a* pattern of neuromuscular response which is built into the organisation of the central nervous system. Thus the sex hormones either contribute towards the accumulation of action-specific energy (Lorenz, 1950) or they facilitate the release, by adequate environmental stimuli, of the "block" which normally holds such energy in check (Tinbergen, 1950).

Nervous and Endocrine Factors in Sensory Perception

There is more to the relationship than this; for although the linkage of specific sensory stimuli with the appropriate behaviour pattern is, like the motor response itself, innate in both sexes (Stone, 1926; Beach, 1940) there are sex differences both in the nature of the sensory stimuli which release *the mating response, and in the neural mechanisms* used for the integration of these stimuli. In the male rat, no single modality of sensation is adequate to arouse the copulatory response (Stone, 1922; Beach, *1942d*; *1944a*), the exciting stimulus being a configurational pattern built up of sensations, which are, initially at least, received by the distance receptors (Beach, 1944*a*). Cortical lesions tend to prevent the response from taking place (presumably by interfering with the

play a significant part in female sex behaviour (Brooks, 1937), the full development of which seems to depend on the stimulation of contact receptors; and destruction of the cerebral cortex, *instead of preventing the initiation of the response* as in the male, results in the dissociation of the complete copulatory pattern into its separate components (Stone, 1938; Beach, *1943*; *1944b*).

Since reversal in mating behaviour takes place under the influence of sex hormones it must be assumed that these hormones, in addition to modifying motor activity, are associated with a re-orientation of perceptual mechanisms.

in one of these tests, the body-righting reaction, the gonadotrophin-injected animals performed less well than their controls. This test therefore merits further consideration.

Each animal was held on its back by slightly stretching it from head to tail. It was then released and its performance scored by judging whether the struggling response of the very young rat had been co-ordinated into the smooth rotatory movement characteristic of the mature animal. The scores, expressed as percentages of the total score for a fully co-ordinated reaction, were plotted against age to give a response curve for each class of rat. To facilitate comparison, these curves were then converted into straight lines using the probit transformation (Finney, 1947). Three facts emerged from this treatment of the data: first, the rate at which the response developed was similar in all four groups; second, there was no sex difference; and third, the mean time of appearance of co-ordination was delayed in the gonadotrophin-injected animals. Statistical tests showed that this delay, while of doubtful significance within each sex, was significant at the 5 per cent level when the data for the two sexes were justifiably combined (Fig. 1).

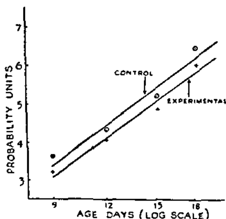


FIG 1. Probit regression lines showing the rate of development of the co-ordination in performing the body righting reaction of normal and gonadotrophin injected rats.

complex patterns of behaviour are built up of simpler components which probably have a lower threshold for hormone facilitation than that of the whole response (Stone, 1939; Berg, 1944; Beach, 1944*c*), it seemed reasonable to expect that the schedule of development of simple automatic behaviour patterns might also be advanced by this treatment. Furthermore, in view of reports of an early difference in the prepubertal activity of male and female (Beach, 1942*a*) it was thought that a closer examination of the development of behaviour in the normal rat might reveal sex differences which had previously been overlooked because quantitative methods had not been used. The results of experiments carried out recently suggest that this is the case.

In the first of these experiments (Eayrs, 1951) the development of automatic behaviour in the rat was studied up to the age of 24 days. Observations were made at intervals of 3 days on groups of 4 litter-mates, each group consisting of 2 males and 2 females. A standard daily injection of 10 I.U. of equine gonadotrophin was given to one animal of each sex in each group. The tests used to study automatic behaviour were based on those used by earlier workers in this field (Small, 1899; Tilney, 1933; Anderson and Patrick, 1934; Biel, 1939), and the observations were placed on a quantitative footing in one of two ways: either by noting the presence or absence of a response at the age tested; or alternatively, by allotting scores to denote the success with which the response was performed. Each rat performed each test once only, and the experimental data were therefore compiled from the performances of different individuals at each age of testing, and not from successive observations on the same rats.

In all those aspects of development in the rat which occur during the first 15 days of life, such as the maturation of posture and locomotor ability, the body-righting reaction, the waning of the grasp reflex, the disappearance of the early convulsive response to painful stimulus, and the appearance and subsequent modification of the "startle" response to mild tactile and sharp auditory stimuli, the plotting of response curves and application of statistical methods did not reveal any significant difference between the sexes. However,

in one of these tests, the body-righting reaction, the gonadotrophin-injected animals performed less well than their controls. This test therefore merits further consideration.

Each animal was held on its back by slightly stretching it from head to tail. It was then released and its performance scored by judging whether the struggling response of the very young rat had been co-ordinated into the smooth rotatory movement characteristic of the mature animal. The scores, expressed as percentages of the total score for a fully co-ordinated reaction, were plotted against age to give a response curve for each class of rat. To facilitate comparison, these curves were then converted into straight lines using the probit transformation (Finney, 1947). Three facts emerged from this treatment of the data: first, the rate at which the response developed was similar in all four groups; second, there was no sex difference; and third, the mean time of appearance of co-ordination was delayed in the gonadotrophin-injected animals. Statistical tests showed that this delay, while of doubtful significance within each sex, was significant at the 5 per cent level when the data for the two sexes were justifiably combined (Fig. 1).

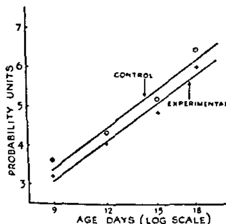


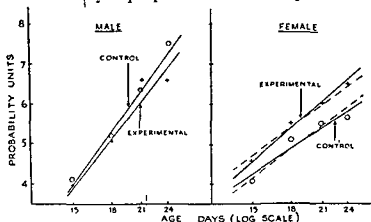
FIG. 1. Probit regression lines showing the rate of development of the co-ordination in performing the body righting reaction of normal and gonadotrophin injected rats

Only two of the responses tested were found to mature later than the 15th day of age: the air-righting reaction and the placing reflex. In both, there was a sex difference. The *maturation of the air-righting reaction* was assessed by dropping the rat, back downwards, from a height of 10 ins., and scoring its performance according to its landing position—back, side or feet. The response matures so quickly that tests at 3-day intervals did not produce enough data to plot adequate response curves, but inspection of the results showed that the mean point on the curves should occur on or after the 16th day. Accordingly, the results for the 15th day were analysed statistically and showed that the male matures significantly earlier than the female. Treatment with gonadotrophin was without effect on either sex.

The time of first appearance of the placing reflex was studied by touching the rat's chin against the edge of the bench. The mature rat responds instantly by placing both its forepaws on the bench on either side of its chin. The percentage of rats giving this response at each age was analysed by the method of probits giving the regressions drawn in Fig. 2. These show firstly, that the response matures earlier and more quickly in the male than in the female. In the second place, the administration of gonadotrophin was without effect in the male, but in the female advanced the mean time at which the reflex emerged.

The varied nature of these results made it unlikely that any single process could account for all, and it was thought that differences both in the normal rates of maturation of the ovary and testis and in their reactivity to gonadotrophin during prepubertal life (Price and Ortiz, 1944; Price, 1947) might explain some of the results. Accordingly the experimental findings were correlated with changes in the appearance of the reproductive organs. Histological examination showed that the secretory activity of the gonads of both sexes was stimulated very early by giving gonadotrophin. Premature secretion of sex hormones therefore does not appear to influence maturation of automatic behaviour during the first 15 days of life, although the inferior co-ordination of the experimental animals in body-righting shows that sex hormones can, even at this early age, influence the performance

of an innate response. This may be due to a weakening in the control of the spinal cord by the mesencephalic centres (Rademaker, 1931) responsible for the mediation of the response, a hypothesis suggested by the chronaximetric studies of Chauchard (1943), which showed that the changes in the excitability of peripheral nerve following treatment of



[illegible]

the female.

the animal with sex hormone are consonant with the effects of withdrawing the influence of supra-segmental nervous structures (Lapique, 1936). A similar conclusion was reache [illegible] ening [illegible]

In c [illegible] ment of the two sexes during the first 15 days, the male developed precociously in later life. Since neither the air righting response [illegible] both presumably [illegible] new integrative c [illegible] case of the placing reflex which depends on the integrity of a small region of frontal cortex (Brooks, 1933) in which the histological changes characteristic of maturation can be seen

at the time when the reflex first appears (Tilney, 1933). It is conceivable therefore that androgen facilitates the final maturation of nervous centres which are refractory to the influence of steroid hormones during the first days of life (Wilson and Young, 1941), a concept which has been discussed by Kollros (1943) in relation to the influence of thyroid hormone on the maturation of reflex centres in amphibia. Possibly the accelerated appearance of the placing reflex in the gonadotrophin-injected female can be explained in the same way, for the injection of equine gonadotrophin into immature female rats is associated with the secretion of androgenic substances by the ovary (Bradbury and Gaensbauer, 1939; Greene and Burrill, 1939; Pfeiffer and Hooker, 1942). The histological appearance of the reproductive tracts of the older females used in this experiment was consistent with this interpretation.

Sex Hormones and Cortical Function

The experiments so far described have been concerned with the development of the rat up to the 24th day of age. Watson (1903) believed that at this age the rat had attained maturity in all forms of behaviour other than those whose appearance is specifically associated with sexual maturation. This view has been questioned by Maier (1932*a*) who found that prepubertal rats performed less well than adults in situations which demanded "reasoning".

Reasoning, in Maier's sense, is not the same thing as learning (Maier, 1932*b*; Campbell, 1935). The learning process involves the permanent association of two or more experiences which have been both spatially and temporally contiguous in the past history of the individual. In reasoning, however, the experiences which are associated have never been present together in the animal's history, the process implying the association of a current experience with the symbolic representation of a past experience. The capacity to reason, and the factors which modify it have been extensively studied by the use of the three-table apparatus (Maier, 1932*b*; Wentworth, 1936; Loevinger, 1938; Wolfe and Spragg, 1934). This apparatus has recently been used to find what changes take place in reasoning ability as the

young rat reaches puberty; whether these changes are modified by a precocious puberty; and whether there are any sex differences (Hanson, 1949).

[illegible]

The rats used, between 30 and 90 days old, were divided into three groups according to age. At the start of the experiment the rats in each group were aged 30 days (prepubertal) 50 days (pubertal) and 70 days (postpubertal) respectively. A fourth group of 30 day old rats which had been brought to a precocious puberty with equine gonadotrophin was also tested. Suitably motivated, the rats were allowed to explore the apparatus for an hour each day for 6 days, this exploration constituting the first experience. The second, and non-contiguous, experience was introduced during the testing period only, and consisted of placing the rat on one of the tables with some food. The rat was then removed and placed on one of the other tables, its powers of reasoning being tested by its ability to combine its recent experience of the presence of food on one of the tables with its past experience of the apparatus as a whole and so, at the centre, to make the correct choice of pathway leading to the food.

The results of this experiment (Table I) seemed at first sight to confirm Maier's (1932*a*) observation that prepubertal rats are less successful than adults in solving the problem, for in the normal groups there was a clear gradient of increasing success with advancing age. At the same time, in all groups the males performed more successfully than the females, and although this difference was not [illegible]

[illegible]

tory action of androgen upon cortical processes, already suggested as a result of the experiments on the placing reflex. None the less it was apparent that the relationship between sex hormones and the functioning of the central nervous system was not a simple and direct one; for although the precociously pubertal rats performed much better than normal rats of the same age tested in the first group they were no better than their saline-injected (and therefore

prepubertal) controls which did as well as if not better than adults. Moreover, there was no sex difference within this group of rats.

Table I

SUCCESS INDICES IN PERFORMING THREE-TABLE TEST
(*Modified from Hanson, D A.*, 1949)

Group	Age (days)	Male	Female
1	30–50 (prepubertal)	41 6 (10)	34 8 (11)
2	50–70 (pubertal)	47·6 (14)	31·6 (19)
3	70–90 (postpubertal)	66 7 (12)	50·0 (10)
4 (a)	30–50 (gonadotrophin treated—pubertal)	75·0 (6)	61·0 (6)
(b)	30–50 (saline injected)	77·8 (6)	77·8 (6)

The success index is the difference between correct and incorrect responses expressed as a percentage of the total number of responses (Maier, 1934)
The figures in parenthesis are the number of rats tested.

A number of factors led Hanson to suggest that the high measure of success of the precocious animals was due to their quicker emotional adjustment to the experimental situation. This he attributed partly to the fact that these rats were tested after experience had been gained in handling the other groups, and partly to their having been regularly handled during the course of their daily injection for some days before testing began. Hence when emotional adjustment is complete, prepubertal animals can " reason " as well as adults; when it is incomplete their performance is inferior. The differences between the sexes may be explained on similar grounds. *It is probable that the activity of the sex hormones* is responsible for the age and sex differences observed, although whether the influence of these hormones is upon the rate of adjustment to the environment, or upon the neural processes concerned in solving the problem or again, whether the sex difference is due to a facilitatory effect by androgen or to an inhibitory one by œstrogen is yet undetermined.

Summary and Conclusions

At the start of this discussion some of the factors were considered which underlie the sex differences observed in the more complicated patterns of instinctive behaviour. New evidence has been presented which shows that, in so far as the male matures earlier, a sex difference is also present in the prepubertal maturation of simpler forms of neuro-muscular response. This does not appear before 15 days old, at which time secretion by the testis seems to be more advanced than that by the ovary. This and other evidence suggests that androgen may in some way advance the time at which previously non-functional centres begin to function. The fact that the experimental stimulation of gonadal secretion failed to produce a similar sex difference during the first 15 days of life suggests that nervous centres are refractory to the influence of sex hormone until an adequate stage of maturity is reached.

A similar sex difference in the performance of a response believed to demand the use of reasoning ability can be traced through puberty to adult life. Although this may be due primarily to dissimilarity in the properties of androgen and œstrogen in relation to cortical function, it is more likely that the difference is secondary to the influence of sex hormones upon the mechanisms responsible for somatic and autonomic adjustment to an environmental situation.

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DISCUSSION

BEACH: In your test of the righting response of the very young animals, what was the volume of the injected material?

EAYRS: We injected them from the first day after birth with 0·1 ml.

BEACH: Did you have control injections in this experiment?

EAYRS: Yes.

BEACH: When you discussed emotional adjustment, this came into your presentation as though it had been a casual observation to which you went back after your results were not what you expected them to be.

EAYRS: That is true. Those observations were made by a colleague of mine; he made certain incidental observations on emotional adjustment which convinced him that the young animals which he was [illegible]

apparatus he used.

HAMMOND: You are dealing with the question of development here in the young animal Have you any control of weight and growth as they were reared?

EAYRS: Yes. The groups of rats used consisted of littermates which were raised side by side by the same mother. An analysis was made of the body weights of the experimental and control animals and this showed that there was no statistically significant difference between them at ages ranging from 6 to 24 days The only outstanding difference was that the females were, as is pretty common, a little smaller than the males.

HAMMOND. I was wondering whether your effect might be due to that fact. Have you tried making your females heavier than your males by better nutrition?

EAYRS No.

HAMMOND: In morphological characters, for example, we can make male and female equal by reducing the plane of nutrition. Conversely, one can increase the morphological difference between the sexes by an increase in the plane of nutrition.

CLEGHORN: Could you not easily control that by comparing different groups of males in different litters with females of comparatively the same weight in other litters? I should think there would be sufficient variation to permit that.

EAYRS: That, I believe, has been taken into account by the fact that we used a wide variety of different weights of females and males. We used many females that were equivalent to or exceeded the weight of many of the males of other litters.

RICHTER What is the difference in weight between males and females at 24 days?

EAYRS: Between 1 and 2 grams, out of 35-40 grams.

DEMPSEY Mr. Eayrs mentioned the nucleolar satellite, and I thought he might be interested [illegible] made at the

quite a lot of talk, and a hasty poll of the people I met in the hallway indicated that at least half a dozen people had attempted to repeat Barr's work in their own laboratories and had failed to do so, but had not bothered to report their failure. So apparently there must be a difference between United States cats and Canadian cats

ZUCKERMAN: It has been demonstrated in this country.

EAYRS: I asked Barr about our failure to find the nucleolar satellite in rats, and he admitted that he couldn't show it himself in rodents. There is too much fragmented chromatin in the nucleus for one to be certain which was the satellite But I have not previously heard it suggested that this sex difference was not a true bill.

DEMPSEY: One can demonstrate very beautiful and clear satellites in the male cat. I have seen some that I can't distinguish from the female variety.

EAYRS: I think that it is admitted that there are some satellites in the male, but the incidence in the female is much greater.

DEMPSEY Yes, and also the size and general appearance was much more beautiful in the female.

ZUCKERMAN: During the period of training, when the young rats became habituated and the control injection rats behaved just as well as the rats which were given the hormones, is there any possibility that the adrenals mediate the process of habituation? The continued stress of injection might cause the production of some androgen or of more cortical hormone.

PINCUS: I think it was shown by Thorn originally that the difficulty with daily injection of a rat is that handling alone stimulates the adrenal cortex.

CLEGHORN: I think I can answer that, because we were interested in studying some effects of adrenaline and noradrenaline in rats, and if one handles the rat and accommodates it to being handled gently, one can get to a point where there is no eosinophil drop.

Pincus: How long does that take?

Cleghorn: I don't really know, but I think that in the series McRae was doing he accommodated them in less than 20 days.

Browne: I wondered about your animal colony, because the condition of the colony very frequently habituates animals ordinarily, apart from experiments. Is it one of the colonies in which one could put one's hand into a cage with impunity?

Eayrs: All rats in our colony can be handled without gloves by most people.

Zuckerman: We have some evidence that as a result of handling the first œstrus occurs precociously in rats.

THE INFLUENCE OF SEX HORMONES ON THE PERFORMANCE OF A LEARNED RESPONSE

S. ZUCKERMAN

IN SO FAR as the body manifests "sexual dimorphism" both in structure and behaviour, it is reasonable to suppose that the genetic and hormonal factors which underlie sexual differences in the first are also responsible, directly or indirectly, for sexual differences in the second, and in particular for sexual differences in innate patterns of behaviour, whatever the time of their appearance in the individual.

By means of various experimental procedures, we can transform, neutralise or fortify patterns of innate behaviour, and by so doing we widen our knowledge of these phenomena. Whether by so doing we also extend our understanding of the underlying physiological processes, or whether such an understanding ultimately depends on the unravelling of the factors responsible for the sexual differentiation of structure and nervous organisation, will probably emerge from some of the papers to be presented at this Colloquium.

Learned behaviour is in a different category from innate behaviour, for learning is essentially a cortical phenomenon. In the preceding paper Eayrs has shown that there are sex differences in the performance by the rat of two presumedly cortically-mediated reactions—the placing reflex, and an example, simple though it be, of so-called "reasoning" (Hanson, 1949)—and has also shown that these reactions can be influenced by sex hormones. The male rat is more precocious than the female in both of these forms of behaviour, an observation which Eayrs suggests may be due to a facilitating effect of androgen upon the central nervous system. The experiment reported in the present paper takes the matter a step further, in so far as it shows that œstrogen may retard or inhibit a learned reaction, and that this effect may be neutralized by means of androgen. The

experiment is not new, but it is nevertheless relevant to our discussion.

It is also relevant to a not infrequently posed and mundane question—"Are men superior in intelligence to women?" No layman's answer to this question can fail to be conditioned by prejudice if account is not taken of the historical and social circumstances by which the sexes are constrained. Equally, no experimental psychologist can fail to be somewhat equivocal in his reply—for his answer depends on the kind of observation which he regards as connoting learning or intelligence.

For the purpose of the present discussion I propose classifying observations on the subject under three heads:

(1) the occurrence of sex differences in learning,
(2) the influence of gonadectomy on learning,
(3) the effect of sex hormones on learned responses.

Sex differences in learning

The rat is the only species in which the problem of sex differences in learning capacity appears to have be[illegible]

of the many tests that have been made largely reflect the ways in which the enquiries were carried out, and that they are consequently of little value. In a well-conceived experiment of their own, in which an attempt was made to control as many extraneous factors as possible, and in which simple mazes and simple multiple light discrimination boxes were used (six mazes and discrimination boxes in all), these two workers found that male rats display neither more nor less learning ability than female rats, and that their learning scores are neither more nor less variable. Tomilin and Stone suggest that a similar conclusion probably applies to learning situations different from those which they investigated, when full allowances are made for such sex differences in temperamental traits, etc., as may influence the indices of learning ability used in standard laboratory situations.

To the best of my knowledge Tomilin and Stone's conclusion has not been materially affected by any observations made

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that the response to conditioned stimuli is strengthened during œstrus in the bitch, and weakened after spaying, an observation which could be taken to imply that œstrogen may facilitate learning; while in an extensive study of the rat, Ball (1926) failed to find any difference in maze-learning during different stages of the œstrus cycle. Observations made in my own laboratory suggest that sex hormones can influence a learned reaction.

The apparatus used in this study (Douglas, Hanson and Zuckerman, 1948) was taken from Hull (1934), and consisted of a straight wooden alley, divided into five 3 ft. sections (Fig. 1). These were interchangeable and, when joined

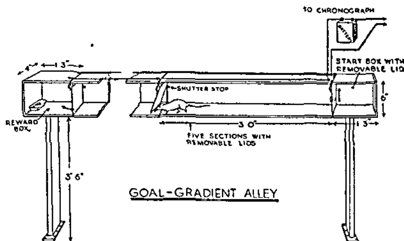


FIG. 1. Goal-gradient alley.

together, provided a 15 ft. enclosed tunnel 4 in. × 6 in. in cross-section, the roof of which could also be removed in sections. At each end of the alley was a short extra section, 1 ft. 3 in. in length, serving as starting and reward boxes. The inner walls of the sections and boxes were painted black, and the whole alley was mounted on an iron frame 3 ft. 6 in. from the floor.

The starting box was closed by a lid, and access to the first section of the alley was by a sliding door which was lifted vertically by hand. There was no lid to the reward box.

Effects of castration on learning

Views about the effects of castration on learning are every bit as equivocal as those which relate to sexual differences in the capacity to learn. Arkhangelskii (1931) suggested that pre-pubertal castration in the dog prevents the establishment of conditioned reflexes, because of the non-facilitation of cortical function by androgenic hormone. Ten years later Anderson (1941) reported that post-pubertal castration in dogs is associated with a slightly weaker salivary response to conditioned stimuli. Tsai (1930), who investigated rats, reached a not dissimilar view, and reported that removal of one or both testes results in a poorer performance in manipulating a problem box or in learning a maze. In his view, semi-castrates are intermediate in performance between normals and fully-castrated animals. These results are, however, controverted by the observations of Tuttle and Dykshorn (1929), who found no difference in the number of errors and the times taken by castrated rats and litter-mate controls in maze-learning during pre-pubertal life, when activity levels are kept similar; and also by those of Commins (1932) and Stone and Commins (1936), who found no differences between the ability of castrate and normal rats to re-learn a maze after a year. These negative experiments appear to have been better controlled than those which suggested a difference in the performance of castrated and normal animals, and on balance it seems safer to accept the conclusion to which they point. Such a conclusion would certainly conform with observations on human beings. Whatever the temperamental effects of gonadectomy in man, there is no indication that the operation is ever associated with any subsequent deterioration in the intellectual capacity of the individuals concerned (e.g., Pittard, 1934).

Effect of sex hormones on learning

Information about the effect of sex hormones on learning is also indecisive. According to Kreps (1923), a previously-established conditioned reflex in the dog is disturbed during heat, while Vanderplank (1938) reports that in the rudd œstrogen inhibits a conditioned response (flight reaction) to a visual stimulus. Conversely, Anderson (1941) suggests

goal-gradient alley exhibits the characteristics which Hull [illegible]

whole course. A gradient appears only when trials are "massed" (i.e., rapidly repeated). Drew therefore regards a gradient as something superimposed on a learned response in the presence of factors that tend to weaken and inhibit the connection between stimulus and response at the moment of response (e.g., rapid repetition of runs; satiation; non- [illegible]

one run every twelve hours. Complete training was marked by a small trial-to-trial variation in time for individual animals, and an attempt was made to train all animals to a mean running speed somewhere near the population mean. Motivation was carefully controlled by adjusting the amount of food given to each animal, in order to avoid masking subsequent alterations in the response.

At the end of training, the animals were divided into four groups of thirteen. Further running tests were then carried out and statistical analysis showed that there were no [illegible]

Gp. I. 0.1 cc. arachis oil.
II. 20 µg œstradiol benzoate in 0.1 cc. oil.
III. 20 µg œstradiol benzoate and 200 α testosterone propionate in 0.4 cc. oil.
IV. 20 µg œstradiol benzoate in 0.1 cc. oil and 1 mg. testosterone propionate in 0.4 cc. oil.

Each animal's running time was then tested every twelve hours from the 6th to the 114th hour.

The results were clear-cut (Figs. 2 and 3). The group-mean running times for the whole alley of the animals in groups II and III were practically identical, as were also those of groups I and IV. The mean running-time of groups II and III were greater than those of control group I at each

The five sections and reward box were divided from each other by light, wooden, one-way swing doors. The opening of the sliding door at the start, and of the swing doors at the end of each section, were recorded electrically on a chronograph run from a constant speed motor, giving a paper speed of 1.1 cm./sec. The chronograph was remotely controlled, and was not housed in the same room as the alley.

The object of the experiment was to train spayed rats to run the alley, and after training to discover whether their speed of running, either of the whole alley or of its different sections, was materially affected by means of sex hormones. During the process of learning to run the alley, rats run faster near the end of the run, i.e., nearer the food box, than they do in the starting box or first section. When learning is complete, the speed is the same in all sections of the alley.

The theoretical basis of the goal-gradient alley is the hypothesis, derived by Hull (1932) from a number of observations on maze-learning, that there is an "excitatory gradient" in motivation and strength of conditioning, the gradient increasing in intensity from the beginning of the maze to the food box; in other words, that there is a progressive increase in the strength of excitation as performance proceeds. Rats under training in the goal-gradient alley can be expected to, and in fact do, run faster nearer the food box than the starting box. In Hull's language, there is a speed-of-locomotion gradient which parallels an assumed goal-excitatory-gradient. But when learning is complete, as already observed, the speed of running is the same in all sections of the alley. On the other hand, inhibition of the learned response by satiation or non-reward causes a return of the excitatory gradient—as is shown by an increase in the time taken by the animals to run the first part of the alley. This is in accordance with the well-known principle that weakening of a learned response restores to that response the type of behaviour which occurs in the earlier stages of training.

It is not essential here to enter into details about the general theory which Hull put forward in order to explain an excitatory gradient. It is, however, necessary to note that while Drew (1939) has confirmed the fact that running in the

The differences in the behaviour of the four groups could not have been due to the oil in which the hormone was administered, since group I acted as a control for group II, and group IV for group III. This experiment shows therefore that œstradiol benzoate is able to cause a response decrement of a characteristic type in a previously learned reaction, and that this inhibition can be prevented by testosterone.

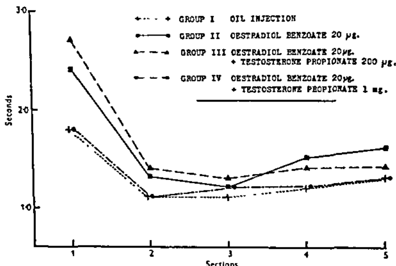


FIG. 3. Mean time taken in each section of alley during experimental period (Reproduced from Douglas, Hanson and Zuckerman, 1948, *J. exp. Biol*, 25, 395.)

The character of the inhibition in this experiment conforms with the observation that weakening of a learned reaction restores to the reaction behaviour which occurred in earlier stages of learning. Thus the increase in total running time was mainly due to slowness in the first two and last sections of the alley. There can be little question, therefore, that œstrogen can inhibit at any rate a simple process of learning, and that the effects of œstrogen can be neutralized by androgen.

12-hourly interval of the 114 hour test, the variance between the four groups after treatment being significantly greater than that within the groups. Owing to the high variance of individual observations, the mean differences between groups I and II and between groups I and III were significant only at 78 and 18 and 78 hours after injection respectively. The high variance of the observations in groups II and III was explained by the fact that the times at which slowing occurred, after injection and the length of time it lasted, varied from animal to animal.

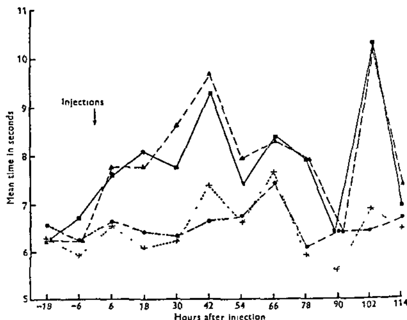


FIG. 2 Group-mean running-times (Reproduced from Douglas, Hanson and Zuckerman, 1948, *J. exp. Biol.*, 25, 395.)
[See Fig. 3 for explanation of symbols]

Groups II and III took longer to run each section of the alley than did control group I. The difference between sections was significant only in the first section for group III and the last section in group II—although it approaches significance in all sections for this group.

inhibition are little more than restatements of the fact that neural action is inhibited, and little useful purpose is likely to be served by speculating here how the observations which I have reported affect general theoretical matters.

There is a further possibility which needs to be considered, namely that the external inhibition occurred as a result of the stroking of the rat's back by the swing doors through which it passed. Such a tactile stimulus would be expected to have a greater effect in an œstrogenized than in a spayed female rat. This possibility could be examined in an experiment similar to the one I have described, but in which the timing [illegible]

[illegible]

The observations which I have reported bear, as I have indicated, on a very wide theme—are there sex-differences in higher cerebral functions such as learning, and can learning be affected by sex-hormones? The answer to this question is clearly yes. But the significance of my observations to the wider question is equally very slight. All that our experiments have done is to provide just another fact—whose interpretation must await a deeper understanding of the matters which are the theme of this Colloquium.

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In our discussion of this result (Douglas *et al.* 1948) we considered the alternative possibilities that the inhibiting effect of œstrogen represented a process of "adaptation" due to the repeated elicitation of the response, or that it was a case of "internal inhibition", due to alteration in the strength of the conditioned stimulus. Both were regarded as excluded by the circumstances of the experiment, and we therefore suggested that œstrogen exercised its effects through "external inhibition"—that is to say, by allowing distracting stimuli to interfere with the learned response.

The possibilities seemed to be that the external inhibition resulted directly from tissues which retain water after œstrogenic stimulation (e.g., the reproductive organs, brain, and other tissues: Zuckerman, Palmer & Hanson, 1950); or indirectly from the effect of such hydration upon other functions of affected tissues (e.g. the effect of hydration on the blood-supply of the brain); or indirectly from the effect of œstrogens upon the balance of autonomic activities. The obvious possibility would seem to be the first, in so far as activation of the genital tract might be expected to alter motivation through a reorientation in the hierarchy of drives. Opposed to any such view is, however, the fact that cyclical "œstrous" variations in the spontaneous activity of the female rat persist after the uterus is removed (Wang, 1923), and that sex behaviour could be induced in a female rat suffering from congenital absence of the reproductive organs (Beach, 1945). A further argument against this suggestion is the fact that changes in behaviour persist, it is believed, long after the water content of the reproductive tissues has been restored to normal.

If we exclude this possibility, the alternative explanation would seem to be some direct pharmacological action of sex hormones on the central nervous system. It is known that caffeine, amphetamine, adrenaline and strychnine all improve performance in maze-learning in one way or other, and that these drugs are cerebral excitants. Œstrogen may, therefore, well be a central depressant, even though the available information does not permit one to see how such a view would fit into any of the classical theories of inhibition. As Freeman (1948) reminds us, however, some theories of

than others, but that the real explanation of central inhibition is still wrapped in mystery. Is that correct Dr. Richter?

RICHTER: As far as I am concerned, certainly.

BROWNE: Has it anything to do with the concept of inhibition as developed by the conditioned reflex people?

ZUCKERMAN: Yes. If we accept the theory that inhibition is due to a change in excitability at the cell membrane, we would be entitled to assume that œstrogen exerts some effect at the synapse, and that we are dealing with a cortically mediated response.

BROWNE: Does that mean that under the influence of œstrogens the afferent stimulus will be irradiated more generally, will not proceed by certain channels?

EAYRS: I don't think one can answer; it is completely in the realms

nervous system as a whole' for a change in mode of behaviour implies

stimuli which were formerly inadequate to arouse a given response are now able to do so.

ZUCKERMAN: They have to push up a flap The apparatus can be made much more complicated. Drew made his animal cross various

effect of œstradiol then on the time taken from the start to the finish?

ZUCKERMAN: I do not know, but I should imagine so.

BEACH. I suspect that the doors are not essential.

EAYRS: The doors are not essential to the goal gradient, but they may be germane to the problem of the source of inhibition with œstrogen.

ZUCKERMAN: That is so.

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DISCUSSION

[illegible] has anything to do with this. [illegible] und [illegible] oid [illegible] me hyper-thyrotic more frequently than males. Œstrone can decrease thyroid activity by 60–80 per cent.

ZUCKERMAN: Are you suggesting that the effect of the sex-hormone is mediated by way of the thyroid?

REISS: It might be. Besides, œstrone has a very strong influence on the blood content of the brain. It is possible that œstrone affects brain function in this way, particularly in rats.

BROWNE: You implied, I gather, that the learning was not in the nature of an inhibition?

ZUCKERMAN There are two views on this point. According to Hull, there is an excitatory gradient—the closer the animal gets to its goal, the greater the excitation, and the more rapid the speed of its performance. In the first instance he based this view not upon experiments in the goal-gradient alley, but upon the analysis of a number of other kinds of learning experiments (e g. maze-running). He then put forward the general hypothesis that there is a goal-gradient in learning, and from it he derived a number of deductions which he and his pupils set out to test, using the goal-gradient alley. As a result, he arrived at some fairly definite conclusions about the character of learning, in the course of this work confirming the existence of an initial gradient.

Drew repeated this work, but instead of observing a goal-gradient at the start, he made the reverse observation that unless you train the rat to run repeated series of trials in close succession, no gradient is evident. Drew then referred the gradient to the operation of some process of inhibition.

BROWNE: What is meant by a "process of inhibition"?

ZUCKERMAN I understand that theories of inhibition are fairly numerous, and that there are one or two explanations better favoured

PATTERNS OF MALE SEX BEHAVIOUR

ARTHUR WALTON

The pattern of behaviour which an animal exhibits in mating, and in courtship and foreplay, is determined to a large extent by the anatomical structure and physiological functioning of the copulatory organs of both sexes. In mammals there is great diversity between species in the structure of the reproductive system, a greater diversity perhaps than that shown by any other system of the body. The male organs are more diverse than those of the female, but since copulation involves both sexes mutually, specific features of the male organs are generally correlated with complementary features in the female.

In the male the greatest diversities are shown in the structure of the penis, and in the development of accessory sexual glands. The differences are reflected in the physiological mechanisms of erection, intromission, and ejaculation, and in the times which these components of the sexual pattern occupy during copulation. Correspondingly, in the female, diversities in the structure of the vagina, cervix and uterus are reflected in the physiological mechanisms of retention of the penis, transport of the seminal fluid within the genital tract, and the times which these components take in copulation. A few examples, drawn from the domesticated animals and laboratory animals with which I have worked, may help to illustrate these generalisations.

Two extreme types of penis which are recognised are the vascular type and the fibro-elastic type. The penis of the horse is a good example of the former. In the non-erect condition it is quite flaccid and withdrawn within the prepuce. Erection and protrusion are effected by gradually increasing tumescence of the erectile tissue in the corpus cavernosum penis. Erection usually takes place rather slowly and seems [illegible]on the continuous reception of erotic stimuli

BEACH: As I understand this interpretation, and as I understand your technique, 90 per cent of the learning as far as pushing doors is concerned had taken place before you started any treatment.

EAYRS: But there remains the possibility of inhibition from, for instance, the touch of the doors along the animals' backs, particularly if the animal is under œstrogenic influence.

BEACH: What do you mean by "inhibition"?

EAYRS: Shall we say it is the result of an external stimulus which distracts attention from the goal. If an animal's back is rubbed when

RICHTER: I have never used these cages. How do you clean them? And do you use the same runway for all the rats, because, after all, these animals treated with different hormones will have different odours. How do you control that?

ZUCKERMAN: It is always the same alley. Hull marked all sections with the same food-smell and tried to eliminate the gradient that way. We followed the same procedure

EAYRS: The question of animal smells was controlled by running the animals under a random series of numbers, so that an œstrogen-treated animal did not always follow a testosterone-treated animal, and so on.

RICHTER: I would think you would have to use separate alleys for the different groups of animals.

ZUCKERMAN: That might be worth trying. The observation I have reported is not very profound. All it represents is a small example of the general proposition that sex hormones have an influence not only upon innate but also on acquired patterns of behaviour.

remarkably erectile and on distension increases in diameter more than two-fold. This has two functions, it aids retention of the penis in the vagina, and it dilates the anterior end of the vagina and cervix, so that the urethral meatus and the os come in close apposition. When ejaculation occurs, in a sequence of four or five spasms, the semen is injected through the os into the uterus. The volume of semen ejaculated by the horse is large (50 to 300 c.c.) and the bulk is derived from well developed accessory glands.

Uterine insemination, or fluid ingress of semen into the uterus, is typical of other species. In my experience of the domesticated animals and common laboratory animals it is characterised by the following:—(1) Peristaltic contraction of the uterine tract, at the time of coitus or shortly after (this is demonstrable in the mare). (2) retention of the penis in the vagina as already described. (3) a relatively large volume of semen, and (4) a cervix which is easily dilated, especially at œstrus.

The bull possesses a typical fibro-elastic penis. It is of narrow diameter and tapers to a blunt end. Due to the marked development of fibrous tissue it is relatively rigid *even in the non-erect condition. The erectile tissue is small* and the penis undergoes little enlargement on erection, although it becomes more rigid. When not protruded from the prepuce, the penis is flexed below the pelvis by contraction of the retractor muscle. Protrusion is effected partly by erection but mostly by relaxation of the retractor muscle and straightening of the sigmoid flexure. In mating there is little or no display. The bull 'tests' the cow by smelling or exploring the vulva with muzzle or tongue and by placing his chin on her tail head; if not in œstrus the cow moves away rapidly, if in œstrus she stands still. The bull often mounts

and protrusion, and these may take place practically simultaneously with mounting. Intromission is effected rapidly, there is a single copulatory thrust of the pelvis, and a single ejaculatory impulse which is reflexly elicited mainly in response to temperature. Insemination is typically vaginal.

during foreplay. Should the stallion mount before the penis is fully erect, copulation is seldom completed, partial erection is lost, and the stallion dismounts. Foreplay appears, therefore, to be an essential accompaniment of vascular erection in this animal. Its actual pattern also is determined by anatomical considerations. In the erect or semi-erect condition the vascular penis of the stallion is extremely vulnerable, especially at the moment of mounting, to a backwardly directed kick from the mare's hind feet. The stallion approaches the mare with caution, curveting, and displaying his crest and mane. Rearing and shying in a circle at a safe distance from the mare, he gradually approaches at the mare muzzle to muzzle, stamping with the forefeet and snorting. If the mare does not vigorously reject or evade these attentions, the stallion then approaches the mare's flank and with neck extended, keeping his hind-quarters well out of the way, he bites playfully at the mare's flank. If the mare is in full œstrus she responds by standing still, straddling the hind limbs, arching the tail and spasmodically erecting the clitoris. If the stallion's penis is now erect, he mounts rapidly and immediately attempts intromission. This is a rather critical moment. If intromission is delayed, even a fully œstrous mare may become restive and actively evade the stallion's further attempts to copulate, and the stallion, himself, may lose erection. Rapid intromission is however aided by the fact that until the penis is actually inside the vagina, only the corpus cavernosum penis is erected, the corpus cavernosum urethræ and the glans are still flaccid.

Introduction of the penis into the vagina has an immediate and very marked restraining effect upon the mare; even an anœstrous mare will stand still when the penis has been inserted, and this response aids retention of the penis in the vagina. In the vagina the penis receives stimulation from friction or pressure caused by the spasmodic pelvic oscillations and thrusts, or from stimuli derived from the temperature of the vagina. Judging from experiments with the artificial vagina, the former are the more important. This stimulation reinforces erection and the corpus cavernosum urethrae and the glans penis erect. In the stallion the glans penis is

within the vagina for a considerable time. Ejaculation is elicited by friction or pressure impulses from the vagina. Temperature alone is not an excitatory stimulus. The artificial vagina will elicit ejaculation over a wide range of temperature, but pressure impulses must be simulated by rhythmically inflating or deflating the lining. Ejaculation involves several peristaltic contractions of the urethra. The accessory glands are well developed and contribute a considerable volume of accessory fluid to the ejaculate. The whole process of ejaculation may be repeated two or three times during copulation. There is a period of rest between each ejaculation but the penis is usually retained within the vagina. Just prior to ejaculation the boar renews pelvic oscillations, but during actual ejaculation makes little muscular movement. Insemination is uterine, and the cervix is easily dilatable. There is some indication that a vaginal plug is formed, from the very copious secretion of the bulbo-urethral glands, which sets to a lumpy glutinous mass, but the functional significance of this is not clear.

Lastly I should mention briefly the laboratory rodents which possess the characteristic feature of the formation of the typical vaginal plug. In these animals, rats, mice and guinea-pigs, a single ejaculatory thrust is made and the penis is immediately withdrawn. By analogy with the bull, one might expect vaginal insemination of a small volume of concentrated semen, but uterine insemination occurs post-coitum, by the formation of the vaginal plug, which physiologically replaces the retained penis, and prevents the escape of semen by the vaginal orifice. The volume of the ejaculate is relatively large and the accessory glands particularly well developed.

I think I have given sufficient examples to show that the anatomical structures and physiological functions of the reproductive organs of both sexes must determine to a very large extent the pattern of behaviour not only in mating but also in foreplay. The pattern is complex and involves many behavioural components: appetitive behaviour, instinct, reflexes, conditioned reflexes, trial and error learning, etc.; and it ends with a typical consummatory act, with release of reaction specific energy.

A small volume of semen is ejaculated (5 c.c.) containing a high concentration of spermatozoa, with little accessory fluid mainly derived from relatively poorly developed seminal vesicles. The penis is not retained in the vagina for more than a few seconds. The cervix of the cow is long and rigid and the lumen tortuous; it is not easily dilated even at œstrous.

I am tempted to speculate that this type of pattern, with little foreplay and with rapid completion of the copulatory act, might be of advantage to animals which in the natural state were subject to attack by predators, since it reduces the time at which an animal is particularly vulnerable. I hope Prof. Hediger will correct me if I am wrong. If correct, however, it suggests that the long time spent in copulation by the only two carnivores which I have studied in the laboratory, namely the dog and the ferret, is also not without significance.

The male reproductive tract of the dog has many interesting anatomical features. The penis is vascular, and erectile tissue is particularly well developed at the posterior end of the glans which forms a rounded enlargement, the bulbus glandis. Part of the corpus cavernosum urethræ is ossified to form the os penis, which is grooved ventrally. In this groove lies the urethra. The bulbus glandis and the os penis are associated with very long retention of the penis in the vagina, where it is held by a marked contraction of this organ. The pressure of the vagina however does not occlude the lumen of the urethra, since this is protected by the groove in the os penis. When the
the penis is retained in the
the corpus cavernosum
causing torsion and tension to be exerted on the retained penis. Ejaculation occurs in this position. Although the accessory glands are poorly developed (the seminal vesicles are absent) a relatively large volume of semen is ejaculated in repeated spasms. Insemination is uterine, peristalsis occurs, and the cervix is easily dilated.

In the boar we have an example of a typically fibro-elastic penis, but in contrast to the bull, the boar mounts the sow prior to protrusion and erection, and the penis is retained

about three to four weeks before mating actually begins, the stags running the hinds and coralling them into little groups, which break up and re-form, and so on. Is all that foreplay?

Hediger: It is a question of terminology. I would call it a part of foreplay. There is no direct relation between the length of the foreplay and the length of the breeding season.

Matthews: The same thing does not apply to the roe deer, because the roe deer lives in family parties all the year round.

Zuckerman: The bucks are together all the time?

Matthews: No, the bucks are separated, each has its own party of does.

Zuckerman: The stags start running the hinds weeks before they attempt mating?

Matthews: No.

Beach: One thing that puzzles me a bit is Dr. Walton's reference to the nature of the dog's ejaculatory behaviour. My own limited observations led me to believe that, in spite of the absence of seminal vesicles, most of the ejaculate is expelled within a second or so, and that continued expulsion during the period of locking was a relatively minor matter. This might be tied up with the fact that pregnancies are possible without locking. Have you any quantitative results that would show when the majority of the ejaculate is expelled?

[illegible]

[illegible]

a relatively dilute suspension first, and then a quite dense opaque suspension almost like ram semen, which you can watch sinking to the bottom of the ejaculate, and then later the type of clearer vesicular secretion again. That's over a period of about 10 minutes locking.

Beach: Did you duplicate the normal copulatory position with the penis pointing backwards?

Hancock: Yes, we use a bitch in heat and as soon as intromission is accomplished, hold the penis backwards in the normal coital position.

Zuckerman: Is there any information about the rate at which the secretions of different accessory glands get mixed up with the semen? For example, the hedgehog has enormous accessory reproductive organs at the height of its season! If I remember correctly, the connecting

It is beyond the scope of this paper to analyse the behavioural components. I have attempted a partial analysis in a previous paper on the bull. (Walton, 1949.)

I wish to emphasise here, however, the fact that in different species, the patterns of behaviour exhibited in mating are very different, and that very many different nervous pathways carrying both excitatory stimuli and motor responses are involved. In one species performance of the pattern may be prevented or disorganised by failure of the exteroceptive sensory mechanisms, in another by failure of the spinal reflex mechanisms, or in another by failure of the autonomic nervous mechanisms. It is probable that the extent to which these mechanisms are energised or facilitated by endocrine factors may also vary in different species. The very complexity of the pattern stimulates interest and invites research.

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DISCUSSION

HEDIGER. There is a general difference between the reproductive patterns of wild and domesticated animals. They are enormously [illegible] by domestication For instance, in the roe deer and in the [illegible] several days In the bison there is a [illegible] aring œstrus. There is normally in the [illegible] tance," each individual is at a certain distance from the next [illegible] al, say perhaps 10 or 20 yards. When bison cattle are in œstrous condition this social distance is [illegible] a typical "œstrous distance," about half [illegible] the side, or just [illegible] ical ceremonial, [illegible], sometimes for [illegible] And this is [illegible]

[illegible] is, that there [illegible] ation between [illegible] mals and their enemies, but I have difficulty in imagining, [illegible], that the zebra, which is related to the domestic horse, has less enemies than, for instance, the [illegible] I doubt whether there is a simple correlation between the [illegible]

[illegible] Professor Hediger, that foreplay is [illegible] the sexes come together for restricted [illegible] the hinds and stags start consorting

about three to four weeks before mating actually begins, the stags running the hinds and corralling them into little groups, which break up and re-form, and so on. Is all that foreplay?

HEDIGER: It is a question of terminology. I would call it a part of foreplay. There is no direct relation between the length of the foreplay and the length of the breeding season.

MATTHEWS: The same thing does not apply to the roe deer, because the roe deer lives in family parties all the year round.

ZUCKERMAN: The bucks are together all the time?

MATTHEWS: No, the bucks are separated, each has its own party of does.

ZUCKERMAN: The stags start running the hinds weeks before they attempt mating?

MATTHEWS. No.

[illegible]

[illegible]

HANCOCK: I have had some experience of collecting from dogs with the artificial vagina for artificial insemination, and in my experience the process of ejaculation is continuous; there is no variation in the rate; the volume per unit time appears to be continuous over the period of collection. I have collected up to 27 ml. at one ejaculation, and I have the impression that the densest fraction comes in the middle. There is a relatively dilute suspension first, and then a quite dense opaque suspension almost like ram semen, which you can watch sinking to the bottom of the ejaculate, and then later the type of clearer vesicular secretion again. That's over a period of about 10 minutes locking.

BEACH: Did you duplicate the normal copulatory position with the penis pointing backwards?

HANCOCK: Yes, we use a bitch in heat and as soon as intromission is accomplished, hold the penis backwards in the normal coital position.

ZUCKERMAN: Is there any information about the rate at which the [illegible]

ducts of different structures, certainly of the external prostate, open independently into the urethra. Do these various accessory glands all discharge their contents at the same time, or do the different glands

[illegible]

instead the physiological respons[illegible] n continues.

[illegible] of the cow. It is very commonly [illegible] at one of the best methods [illegible] ation is to insert your hand into the vagina.

THE EXPERIMENTAL INDUCTION OF SEXUAL BEHAVIOUR BY STEROID HORMONES

EDWARD W. DEMPSEY

THE isolation of the follicular hormone, œstrogen, in the early 1930's, was followed quickly by reports of its efficacy in inducing the morphological and the behavioural characteristics of the œstrous cycle. It soon became apparent, however, that these early reports were over-optimistic, in that the phenomena induced by œstrogen were only partial, or irregular, as compared with their counterpart events during the normal reproductive cycle. Thus, uterine growth may be induced in ovariectomized animals; nevertheless the full differentiation and activity of the uterus requires not only œstrogen, but also progesterone, acting in a synergistic sequence. Similarly, both ovarian steroids are required for full proliferation of the mammary gland. It has recently been shown that vaginal proliferation, long regarded as under only œstrogenic influence and used extensively in the biological assay of œstrogen, is modified by the synergistic action of progesterone, so that the changes in the vaginal smear approach more nearly the sequence of events during œstrus in the normal animal (Ford and Young, 1951). Lastly, the hypothesis of Hisaw that an increasing concentration of œstrogen acted as trigger for the ovulatory release of luteinizing hormone has required modification, since the work of Everett (1951), indicates that simultaneous action of progesterone is involved in the ovulatory mechanism.

The experimental induction of sexual receptivity in ovariectomized rodents follows the general rule that reproductive phenomena respond to the sequential action of œstrogen and progesterone In female guinea pigs, œstrogen alone can induce œstrus, but only in a fraction of the animals. The latent period is long and the response irregular and

atypical. On the contrary, when œstrogen is followed by the administration of progesterone, œstrus is invariably produced, the latent period is short and the duration and quality of the response is normal. The delayed and atypical œstrus induced by œstrogen cannot be improved by higher dosages, nor by altered routes of administration. The œstrogen-progesterone synergy in producing œstrous behaviour was originally worked out for the guinea pig (Dempsey, Hertz and Young, 1936), and has subsequently been substantiated for the rat (Boling and Blandau, 1939), mouse (Ring, 1944), and hamster (Frank and Fraps, 1945).

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[illegible] 269.

DISCUSSION

KLEIN: There is one rodent that you did not mention—the rabbit. I can't say what happens when you inject œstrogen and pro-œsterone simultaneously into a castrated female, but when you inject œstrogen alone [illegible] corpus luteum (a corpus luteu [illegible] you produce a com[illegible] icle bursting and ev[illegible] n a [illegible] animal you can [illegible] ete [illegible] al or synthetic, and I [illegible] te œstrous behaviour in the [illegible] k one must be very careful before drawing a general law in going from the guinea pig, [illegible] and golden hamster Don't forget the rabbit! [illegible]

KLEIN: No. Only after copu[illegible]

HAMMOND: I can add something from the point of view of a comparative study of different species (see Table I).

Table I.

	Horse	*Pig*	*Sheep*	*Cow*
Anterior pituitary .	LH (Progesterone) — FSH (Oestrogen)			
Average duration of œstrus (hours) . .	144	72	24	16
Ovulation (hours in relation to end of heat) . . .	18 before	4 before	0 after	14 after
"Still heats" . .	Rare		Frequent	

The species shown form a series in the duration of œstrus and in the time of ovulation in relation to the end of heat, and also in the frequency of "still heats," that is ovulations without the symptoms of œstrus.

The explanation, as I see it, is to be found in the anterior pituitary, in the relationship between the two main hormones LH (and so progesterone) and FSH (and so œstrogens). It is the balance between these hormones which varies in the different species. So when you administer these hormones to different species you will get different results according to this balance level within the animal concerned.

Browne: Is that the pituitary content of LH and FSH?

Hammond: Yes.

Dempsey. I think perhaps a contrast can be made between the [illegible]

the other events in the reproductive cycle, that it seems to me a re-estimation is required of the possible rôle of progesterone in reproductive events that have formerly been ascribed only to œstrogen.

Klein: In the dog you may be able to get complete œstrous behaviour with œstrogens alone, but often the follicle is partly luteinized before it ruptures. That is the best demonstration that even in the dog some progesterone is necessary for normal œstrus

Beach. An important point would seem to be whether or not in some species progesterone may actually be a heat terminating agent.

DEMPSEY: If I understand you correctly, you mentioned earlier, and Professor Klein also, that heat could be maintained indefinitely, provided that one kept a high titre of œstrogen in these animals. Is that correct?

BEACH: This is true in the case of the spayed cat.

ZUCKERMAN: And in the ferret too.

HAMMOND: In the ferret you can terminate that œstrus at will by putting in tablets of progesterone.

[illegible]

hypothalamus [illegible]
was to produce a localized œstrous state of the hypothalamus [illegible] presence of anœstrous reproductive tracts. Many of the castrated rabbits with implants [illegible] something seemed well until we f[illegible] *without* implants woul[illegible] [illegible] other people have observed that castrate [illegible]

[illegible] verett has observed that [illegible]

[illegible] halamus.

[illegible] hn, we have recently been investigating the question of pituitary transplants, and have evidence—I don't want to go into detail—that the hypothalamus behaves differ- [illegible] will give you [illegible] and LH, and [illegible] or the lack of [illegible] hypothalamus perhaps not only underlying behavioural reactions [illegible] lso controlling the various amounts of gonadotrophin secreted in the two sexes.

[illegible] When you want to test the action of œstrogenic substances [illegible] You [illegible]

HAMMOND. It is the effect of the critical balance that [illegible] get that balance between the two hormones, œstrogenic effects are produced.

KLEIN: Yes.

HARRIS: My rabbits that copulated have had completely atrophied uteri There wasn't enough œstrogen apparently to produce any development there. Are you suggesting that there was sufficient œstrogen to activate the neural mechanism but not the peripheral tract?

KLEIN: That may happen.

ZUCKERMAN: Dr. Beach referred to the possibility that one of the functions of progesterone may be to terminate œstrus. In primates where the sex skin swells, all fluid in the skin is liberated when the corpus luteum starts operating. This does not, however, mean that œstrus has ceased, because the females will still mate, although not as frequently as when the sex skin is swollen Have you made any observations of a similar kind in your earlier work?

DEMPSEY: I was interested in the effect of progesterone in terminating œstrus for this reason I mentioned that a large injection of œstrogen alone in the guinea pig does sometimes lead to prolonged œstrus. If one gives a large injection of œstrogen and then follows it at the appropriate time with progesterone, the œstrus is aborted in the sense that it now occupies its normal duration, that is it stops after about

is not exactly the same as it is on the peripheral tissues. It is necessary to build the central nervous system mechanism up from zero and trip it off and do it over again to get the normal sequence.

KLEIN: The meaning of œstrogen is: something that raises an œstrus. I don't think it must be something which cornifies the vaginal epithelium or causes a uterine reaction.

HAMMOND. Are there any experiments on hypophysectomized animals? Do they mate without added hormones?

BEACH. All of the evidence with which I am familiar is negative.

HAMMOND: If not, it may be possible that the gonadotrophic hormones act on other glands than the ovary to produce œstrogens and progesterone.

could not get any evidence of responsiveness to œstrogen in the intact non-parous female which had not shown heat up to $2\frac{1}{2}$ years.

[illegible]

in the non-breeding season we get exactly the same effect. It is only after the second injection 12 days later, when there is a corpus luteum already present, a basis of progesterone, that you get œstrus. The same thing happens in the young cow. If you inject anterior pituitary FSH into a calf a month old, you will get follicles formed, there is any amount of œstrogen, but the animal does not come on heat. It is only after you get an ovulation, and there is a basis of progesterone that you get heat.

HANCOCK: You still have the absence of progesterone in the spayed animal of course.

FOLLEY. The goat resembles the sheep in that it is a seasonal breeder, but in goats injected in the non-breeding season with PMS a good proportion do show heat, moreover there is often ovulation,

HAMMOND: Yes, but their non-breeding season isn't anything like so deep as the non-breeding season in the sheep. If we inject at the beginning of the non-breeding season in the sheep, when there is an old corpus luteum present we do obtain heat

FOLLEY: There are no corpora lutea in the ovary in the anœstrous season?

HAMMOND: No, but there is progesterone in special circumstances in atrophic corpora lutea remnants.

PINCUS: We should be able to make the sheep go into heat immediately then if we give progesterone with œstrogen.

HAMMOND. We can do it by giving testosterone before PMS, which produces œstrogens (Robinson, J., 1950, *J. agr Sci*, 40, 275).

ON THE EXCRETION OF NEUTRAL STEROIDS IN THE URINE OF NORMAL AND INFERTILE BULLS

P. MESCHAKS

DURING the last 20 years, investigations on infertility in bulls have been carried out at the Veterinary College, Stockholm. This work has consisted of comparative morphological studies of changes in the sperm picture and testicles of bulls with reduced fertility or complete sterility (Lagerlöf, 1934), and extensive investigations of hypoplasia of the gonads in male and female animals of the Swedish highland breed (Ericsson, 1943, and Lagerlof, 1939).

As all the most valuable bulls in Sweden are insured with one big insurance company, it has been possible to use for scientific investigations all the bulls disposed of because of infertility (Lagerlof, 1950). To attempt to find out the importance of hereditary factors in certain types of sterility, investigations are in progress concerning the effect of feeding intensity on serving ability and spermatogenesis in bulls. In these experiments, one-egg twins are being used (Bane, 1949, Hansson and Bane, 1949).

My task in this team has been to study the excretion of neutral steroids in the urine of normal and infertile bulls. Some results of this work, which has been going on since 1945, have been published earlier (Meschaks, 1948, 1950, and Garm and Meschaks, 1949).

Material

The bulls used [illegible]

I. Twenty-f [illegible]

II. Seventee [illegible]
of ability to serve. All were of the Swedish Red Breed.

III. Nine bulls with considerable disturbance in spermatogenesis. Six were Swedish Red and three Friesian.

IV. Four bulls with combined disturbance in spermatogenesis and ability to serve.

The results obtained so far, show that the greatest frequency of sterility appears in the first year of service, i.e., at the age of 18–30 months. This and other facts (the results of investigations on one-egg twins, etc.) show definitely that in *most cases sterility in young bulls is due to an inborn tendency* towards sexual disturbances as a result of an inherited weak *endocrine constitution. There are, of course,* also other causes of infertility in young bulls.

Only bulls in which the infertility, in all probability, was caused by inherited factors, are included in this investigation. *This report includes only bulls younger than 36 months.*

After having been in regular service, the bulls appeared *either to be unable to serve or to produce infertile sperm,* and were therefore sent to the Veterinary College, where they were *kept for several months. During this period they* were subjected to thorough clinical examination, and after *slaughter the endocrine and sexual organs* were examined histologically. The results of these researches will be *published later by Lagerlof and co-workers.*

Methods

The urine samples were taken daily between 8 a.m. and 10 *a.m. and analysed during the same day.*

As shown by Koch (1942) and others, the urinary excre*tion of steroids in man may vary from day to day.* To avoid such variations the urine samples were taken for eight, ten or more consecutive days, and the mean values of the determinations were used.

Twenty to 50 urine samples were taken from some bulls, and examination of the results showed that there was some constancy in the values of steroids excreted. The average excretion of eight to ten consecutive samples seems to be typical for the animal concerned.

As it was not possible to collect 24-hour samples, the specific gravity of the urine was taken into consideration in calculating the neutral steroid content. Dingemanse *et al.* (193

Koch (1942), Garm and Meschaks (1949) and others, showed that the biological activity of the urinary steroids in bulls' urine is much lower than that in man, and thus less practicable for quantitative determinations. For this purpose two colorimetric methods, those of Pincus (1943) and Zimmermann (1935), were therefore applied.

Pincus' method: With antimony trichloride reagent, colourless crude extracts of bulls' urine develop a red-violet colour with two absorption maxima at spectral-filter 47 and 53 in the Pulfrich-photometer.

After Girard's separation, these colour-giving substances appeared as non-ketones, but after succinic anhydride separation as alcoholic non-ketones.

The ketonic fraction in this reaction develops only a feeble red colour.

An exception is the extract of pregnant mares' urine which develops a blue colour with two absorption maxima at S–53 and S–61 (approximately). These steroids, which appear to be alcoholic non-ketones, are excreted in the urine from about the 120th day of pregnancy (Meschaks, 1950).

The antimony trichloride reaction is sensitive to impurities, but is very valuable in showing the chemical differences between the urine steroids from man, cattle and horses.

The blue colour reaction which is typical of androsterone did not appear in urine extracts after injection of testosterone propionate in the cow, bull or ram, 1000, 600 and 50 mg. respectively, which indicated that the steroid metabolism in these animals had occurred in a different way.

Zimmermann's method was applied according to the author's prescription of 1944, i.e., using aqueous KOH solution. In this reaction crude urine extracts develop a pink colour with absorption maximum at S–43. A " cleaner " colour is obtained in the ketonic fraction. Girard's separation showed that purified ketosteroids, so-called 17-ketosteroids, are only approximately one-half of the colour activity of the crude extracts. The non-ketonic fraction with Zimmermann's reagent also develops a colour (yellow) with the same intensity as the ketones. It seems that the non-ketonic fraction in bulls' urine contains a greater amount of steroids

than the ketonic fraction. Therefore, the steroids determined with Zimmermann's reagent in crude extracts of bulls' urine should not be called 17-ketosteroids, but " neutral steroids ", to emphasize the differences from the corresponding fraction in human urine.

Results

This paper includes the results of analyses of 538 urine samples from 54 normal and infertile bulls.

Normal bulls (Zimmermann's reaction).

One hundred and twenty-nine samples from 24 normal bulls were analysed. The results are presented in Table I. The table includes only those cases in which seven or more analyses were made.

Table I

Bull	Number of samples	Mg /litre excretion	
		Range	Mean
1	7	18–26	22.3
2	8	18–26	22.0
3	10	18–34	23.8
4	16	14–33	23.3
5	14	15–31	22.5
6	16	18–32	23.4
7	16	13–29	20.9
8	10	15–27	20.4
9	7	17–30	23 0
—	104	——	22.4

The daily variations in steroid excretion were considerable, but the mean values were more constant. Apparently the excretion level in normal bulls, determined in seven, eight or more samples, may be considered typical of the individual. Twenty-five urine samples from one of the bull-stations (15 animals) showed an excretion range of 10 to 28 mg. per litre.

Bulls with lowered ability to serve

This group includes bulls with poor sexual desire and those unable to copulate. One hundred and eighty-seven urine samples from 17 animals were analysed. The results are presented in Table II.

Table II

Bull	Number of samples	Mg /litre excretion	
		Range	Mean
1	12	25–45	34
2	11	14–35	23.7
3	11	20–43	29
4	11	19–52	29
5	5	22–30	26
6	6	19–35	25
7	7	19–38	26
8	12	20–38	27
9	25	19–50	31.3
10	19	16–45	29
11	10	17–33	26
12	8	21–39	29
13	8	23–33	29
14	12	20–38	32
15	10	23–31	27
16	10	19–37	28.5
17	10	20–30	24
—	187	——	27.9

There was no sharp border between the excretion levels in normal and infertile bulls. More striking was the irregular occurrence of excessive excretion up to 40 mg. to 50 mg. per litre.

Bulls with disturbances in spermatogenesis

One hundred and seventy-one urine samples from 9 bulls were analysed. The bulls were able to serve well, sometimes better than normal animals. Four of these bulls showed clear symptoms of testicular hypoplasia. The results are presented in Table III.

Table III

Bull	Number of samples	Mg /litre excretion	
		Range	Mean
1	10	19–44	29.5
2	14	21–43	31
3	50	19–47	33.5
4	20	27–43	33.7
5	23	24–40	34.7
6	10	23–55	36.1
7	20	26–50	39
8	14	28–48	38
9	10	29–43	35.5
—	171	——	34.5

The excretion level in these bulls was higher than in normal animals and higher than in bulls with inability to serve.

Bulls with both inability to serve and disturbance in spermatogenesis

Table IV

Bull	Number of samples	Mg /litre excretion	
		Range	Mean
1	10	22–36	30
2	5	26–37	32
3	10	25–44	32.4
4	26	19–54	36.5
—	51	——	32.7

This group seems to be an intermediate one between the two main groups.

Parallel determinations using Pincus' and Zimmermann's reactions showed that infertile bulls excrete more alcoholic non-ketones than normal bulls. The results are recorded in photometric units. (See Table V).

Table V

Species	Pincus' reaction		Zimmermann's reaction	
	S-43	S-53	S-43	S-53
Normal bulls	0.72	1.24	0.66	0.48
Bulls with sperm-failure	1.14	1.08	1.00	0.70

Bulls with sperm-failure excrete in the urine increased amounts of both ketonic and non-ketonic steroids.

Statistical Analysis

Group	No.	Mean	Standard deviation	Difference
Normal bulls	9	22.7 ± 0.66	1.98	
Bulls unable to serve	14	27.66 ± 1.17	4.38	P 0.002
Bulls with sperm-failure	9	35.24 ± 1.58	4.73	P 0.001

From this calculation it may be concluded that the differences in excretion of neutral steroids in the urine of normal bulls and bulls with lowered ability to serve are statistically significant (P 0.002).

The excretion differences between bulls with inability to serve and bulls with disturbances in spermatogenesis are highly significant (P 0.001).

A comparison between bulls with inability to serve and bulls with disturbances in spermatogenesis is shown in Table VI on the next page.

Table VI

Bull No.	Number of samples	Average Mg /L	Ability to serve	Sperm quality	Testicular hypoplasia
306	11	24	0	—	—
313	10	24	0	—	—
755	6	25	0	—	—
34	5	25	0	—	—
614	7	26	0	—	—
255	10	26	0	—	—
731	12	27	0	—	—
560	10	28	0	—	—
828	10	28.5	0	—	—
36	9	29	0	—	—
37	9	29	0	—	—
29	11	29	0	—	—
30	11	29	0	—	—
749	19	29	0	—	—
41	10	29	—	0	—
862	10	30	0	0	—
18	25	31 3	00	—	—
3	13	30.4	—	00	—
27	12	32	0	—	—
644	5	32	0	0	—
618	10	32.4	0	0	—
307	10	33	0	—	—
344	50	33 5	—	00	Hypoplasia
54	20	33 7	—	00	Hypoplasia
38	23	34.7	—	00	Hypoplasia
562	10	35.5	—	0	—
761	26	36 5	0	0	—
5	10	36.1	—	00	—
18	10	38	—	0	—
39	20	39	—	00	Hypoplasia

0 = lowered activity.
00 = absence of libido or spermatogenesis.

Discussion

All the infertile bulls examined had normally developed sexual organs, which shows that during the period of growth there had been no lack of androgen stimulation.

The mode of erotic activity of androgens is not completely clear. A great number of experiments are published, showing that testosterone and some other steroids possess erotic activity.

Hooker (1944), demonstrated that the appearance of puberty in bulls is not associated with an increased androgen production. He postulates that there is an increase in the sensitivity of the tissues to androgen stimulation.

Since the work of Bochefontain (1876) the existence of a central nervous control of the genital apparatus has been demonstrated.

Harris (1937), Westman and Jacobsohn (1937), Hillarp (1949), and others, have demonstrated the importance of the hypothalamus in genital function.

Denamur and Simonnet (1950) state that the genital instinct is controlled from the hypothalamus.

The anatomical studies of Vazquez-Lopez (1949), and Stöhr (1950), show that very fine abundant nerve fibres innervate the cells of the anterior pituitary gland and other endocrine glands. Thus a direct central nervous regulation of hormone production could be postulated.

Beach (1948), and Seitz (1939), hold that initial failure to mate is due to very low degrees of sensitivity in the nervous mechanisms for sexual arousal. This variable lies in the genetically determined responsiveness of the nervous mechanisms mediating sexual arousal and mating behaviour.

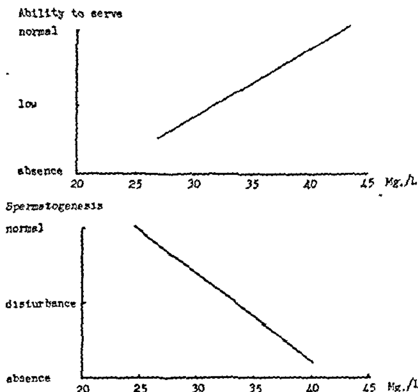
On the other hand, increased production of androgens may depress the spermatogenesis and cause testicular atrophy, as shown by Moore and Price (1932), and others.

Simpson (1948), states as a physiological law in endocrinology that excessive amounts in the blood stream of the specific hormone of an endocrine gland, either endogenous or exogenous, will produce involution or atrophy of the contralateral gland or both glands.

The results of the present investigation show that the 80 young bulls with special forms of infertility excreted more neutral steroids in the urine than normal bulls. The excretion differences between normal bulls, bulls with inability to serve, and bulls with failure in spermatogenesis were statistically significant.

The results indicate that these forms of sterility may be only different degrees of the same condition, attended by increased excretion of neutral steroids in the urine.

The increased production of neutral steroids may be considered to be a compensatory reaction of the central



FIGS. 1 and 2. Graphs showing that with increased neutral steroid excretion in the urine of infertile bulls, the serving ability rises, Fig. 1, and the spermatogenesis falls, Fig. 2.

nervous system, to stimulate the sexual arousal and mating behaviour (Beach).

Libido and ability to copulate increase with the increased excretion of neutral steroids, whereas the spermatogenesis decreases (see Figs. 1 and 2).

The four bulls which showed both lack of libido and disturbances in spermatogenesis excreted in the urine a mean of 32 mg. per litre (51 samples).

The four bulls with symptoms of partial testicular hypoplasia excreted in the urine a mean of 37.7 mg. per litre (131 samples).

The increased excretion of urinary steroids in these infertile bulls is demonstrated by means of both Zimmermann's and Pincus' colour reaction.

The principal activity of the sexual instinct is apparently *to maintain and regulate the sexual drive. If the response of the regulating centre to androgen stimulation is lowered,* the production of sex-hormone can be so high that spermatogenesis suffers, and symptoms of partial testicular hypoplasia may appear (see Table VI).

Summary

1. Zimmermann's and Pincus' colorimetric methods have been used for quantitative determination of the excretion of neutral steroids in the urine of normal and infertile bulls.
2. These two methods show that in young bulls with a special type of sterility there is an increase in the amount of neutral steroids (ketones and non-ketones) excreted in the urine.
3. The differences in excretion between normal bulls, bulls with inability to serve, and bulls with disturbances in spermatogenesis, are statistically significant.
4. With increased excretion of neutral steroids in the urine, the ability to serve increases, whereas spermatogenesis decreases.

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DISCUSSION

PINCUS: The nature of these non-ketonic steroids is still unknown, particularly as far as bulls' urine is concerned. Even in human urine

examined. The average values in the total extract, ketones and non-ketones, separated into alcoholic and non-alcoholic fractions, were the same in both the Zimmermann and Pincus colour reactions. It was striking that the range of values (6) was greater after castration—25–40 and 19–50 mg./l.

As shown by Marker the steers' urine contains no pregnanediol. It was expected that the fraction of alcoholic non-ketones would show changes after castration. That was not the case.

[illegible] the [illegible] uc steroids in human urine at any rate, represent substances of adrenal origin, and for that reason I think that the condition of the adrenals of these animals should be of interest.

BROWNE: What was the method of hydrolysis of the urine?

MESCHAKS: I have used 7 5 per cent (volume per cent) hydrochloric acid. I have tried concentrations of 4 per cent to 15 per cent, and there was no great difference. But using low concentrations of acid, we cannot get out all the steroids because the urine of animals is more alkaline [illegible] st frequency of [illegible] corrected for a [illegible] three years of age I could not find any difference in excretion with age. I have examined bulls more than five years of age, and there appears to be some tendency to lowering of excretion in normal animals, but there was not sufficient material for me to postulate that

BEACH: Dr Meschaks has reported very careful work on the chemical side of the problem. Now I would like to know if he has any more sensitive or delicate behavioural measures than simply "good service," "poor service," "no service."

MESCHAKS. It is quite a difficult question to answer. The degree of inability to serve, the absence of sexual drive or desire I could not show in degrees, but only whether it is present, very poor or absent.

HARRIS. Dr. Meschaks stated in discussing the significance of his results that the endocrine glands were innervated by a multitude of fine nerve fibres. I would like to disagree on that point. I think the evidence shows that the pars distalis of the anterior pituitary and the adrenal cortex lack any innervation, and that other endocrines, such as the gonads and thyroid, will function normally if transplanted, so that any nerve fibres present are of doubtful significance so far as the secretory activity of these glands are concerned.

ZUCKERMAN: This is partly a question of anatomical interpretation, and of Vazquez-Lopez's paper in particular. The nerve fibres which he shows running into the pars distalis, as Dr. Harris correctly points out, have been doubted by one or two other neuroanatomists.

OBSERVATIONS ON REPRODUCTION BEHAVIOUR IN ZOO ANIMALS

H. HEDIGER

The effect of sex hormones being investigated foremost on laboratory and domestic animals, I consider it my task to report upon the behaviour of some wild animals, as they are *observed in the natural state and in zoological gardens.*

The white rat, the white mouse, the guinea-pig, the rabbit and other laboratory animals, they are all—biologically speaking—far from the natural state. They are, in a way, abstract animal species, foreign to nature. The same is true to some extent of the other domestic animals. They are man-made creatures which do not exist in the wild. Therefore, all conclusions drawn from their psychological and physiological behaviour, do not qualify for generalization and transposition to the conditions of wild animals. Wild animals constitute at least 98 per cent of mammals. Apart from a few exceptions, laboratory experiments are concerned with a tiny minority of 2 per cent of mammals.

With reference to reproduction behaviour, we are able to observe in a zoo three contrasting groups of wild animals, viz.:

(1) those in which reproduction is completely arrested by captivity; living in a zoo practically means sterilization for them;

(2) those in which the reproduction pattern is not essentially affected;

(3) those in which the reproduction behaviour is exaggerated in captivity in a striking way.

It may, therefore, be taken for granted that the metabolism of sex hormones is influenced by captivity in different ways.

The following most notorious non-breeders belong to the first group; life in captivity has a sterilizing, or at least a strong *inhibiting effect on them:*

Gorilla (no birth in captivity up till now)
Cheetah „ „ „ „

European otter (no birth in captivity up till now)
Flamingo (only in Hialeah, Miami, Florida, since 1937)
Giant Tortoise (only in India exceptionally).

In regard to the gorilla, it can be said that never so far—with one exception ("Makoko", Bronx Zoo, New York, died 1951)—has a male specimen reached sexual maturity in captivity, although several have lived more than 15 and 20 years. Several cases of fully-grown male gorillas are actually known showing decided genital hypoplasia, e.g., the huge gorilla "Bobby" of the Berlin Zoo (Koch, 1937).

In contrast to the gorilla, the chimpanzee, who is closely related to him, when kept in captivity under the same conditions, is induced to breed without any difficulty. The third generation has already been obtained in the Yerkes Laboratory of Primate Biology of Orange Park (Florida). Obviously, the care of gorillas lacks a decisive factor for reproduction. What this factor is we do not yet know. According to experience gained in zoological gardens, the following factors influence reproduction in wild animals:

(1) Psychology.
  (a) Relation to the space (e.g., depressing effect).
  (b) Relation to individuals of the same species (individual antipathy).
  (c) Relation to other species, above all to men (e.g., continuous excitement by the presence of man as an enemy).
(2) Nutrition.
  (a) Substantial food.
  (b) Vitamins and hormones.
(3) Milieu (environment).
  (a) Inside accommodation (e.g., nesting material).
  (b) Climate, temperature, certain light rays, humidity, etc.
  (c) Geographical position.
(4) Inhibition of locomotion through surgical operations (e.g., by pinioning the wings of birds).

In the case of the gorilla, psychologically conditioned obstacles may be excluded, also environment, and as to nutrition, substantial food. It may be suspected that vitamins and hormones bear responsibility for the genital hypoplasia,

and it is possible that in the wild state, gorillas eat notable quantities of œstrogenous stuff with their foodplants—Bodenheimer (1949), showed lately that the reproduction of a small rodent (*Microtus guentheri*), depends on the intake of certain plants.

They need not act primarily gonadotrophically, but may have—to begin with—a neurotoxical effect operating on the secretion of the hypophysis.

For the cheetah, sterility owing to captivity is probably conditioned by similar reasons as for the gorilla, i.e., by the absence of certain vitamins and hormones. There are certainly other factors for the flamingo and the giant tortoise.

Two types of species breeding in captivity can be distinguished:

(*a*) the fixed type (e.g., polar bear, emu, king-penguin);
(*b*) the variable type (e.g., wild boar, ibex).

The polar bear may be considered as the outstanding example of an animal with a fixed breeding season. Like the European brown bear, this species from the Arctic has its offspring in midwinter. In captivity too, there is hardly any spread of the breeding season to be observed (Hediger, 1950). Some data of polar bear births in the Zurich Zoo, which Director F. Hofmann kindly gave me, confirm this fact:

1937 : 3rd November.	1943 : 27th November.
1939 : 11th November.	1945 : 27th November.
1941 : 5th November.	1947 : 6th December.

The regularity of the two-year cycle and the limited spread of the dates of birth are very characteristic.

Giraffes, on the contrary, are born in every month of the year. Here, however, we must carefully distinguish between the biologically variable type which is not linked to a breeding season in freedom, and the other type which becomes variable in captivity. The variability conditioned by captivity is very often associated with appearances of hypersexuality, to which we will come back later.

In captivity, observation has been made of many representatives of the fixed types which have been geographically moved, so that they prove capable of adaptation to a new seasonal rhythm during the years. This concerns, for example, the European deer, which has been exported to

New Zealand, or the emu, imported from Australia to Europe, the black swan, etc. It must be assumed that reproductive activity for these animals is directed by the seasons, i.e., by temperature, the daily exposure to light, intensity of light or other light factors. According to the species, a certain amount of light can permit or accelerate reproduction, or on the other hand, can also act as inhibitor

Many species, especially of birds, require a considerable amount of light to be able to reproduce at all. This we observed in the Basle Zoo for the Cocatal (*Calopsitta novae-hollandiae*). For many years, a group of these birds was kept in the inside of the bird-house where there was little light. In spite of all care, they never were persuaded to breed. But, after having them moved into an outdoor-cage with strong exposure to light and sun, they regularly brought forth offspring.

In contrast to these birds, there are animals which may be sterilized straightway by an intensive exposure to light, e.g., *Microtus guentheri*, according to Bodenheimer (1949).

There are animals which can come on heat through abundant food only (e.g., the red deer, *Cervus elaphus*); others, on the contrary, through starvation. For these animals (e.g., raccoon, *Procyon lotor*) plentiful food has even [illegible] ns for the reproduction of all animals, especially mammals. Each group may rather respond differently to the various factors. We have to deal here with a heterogeneity of conditions, the effect of which in each single case is often difficult to perceive; what is stimulating for one animal may be inhibiting for another.

Let us have a look at the third group of zoo animals where the reproduction behaviour undergoes an intensification, so that we may speak of hypersexuality. This hypersexuality manifests itself above all in the following ways:

(1) Extension of the rutting season (and breeding time).
(2) Precocious maturity.
(3) Abbreviation of the intervals between births
(4) Increase of the number of young at one litter.

The ibex is a typical example of an animal showing these phenomenona. We have to admit therefore, that its

hormones are strongly affected by captivity. In the wild, the ibex only begins to breed in its third year; in captivity, this may occur as early as $1\frac{1}{2}$ years. This is an obvious precocious maturity. In the natural state the rutting season is concentrated in the months of November and December, whereas it can be spread out in captivity from October to March, or still longer. Normally, in the wild, each female brings forth an offspring only every second year; in captivity, on the other hand, every year. Twins are rarely known in the natural state, but fairly often in captivity. Similar changes have occurred in the elk (*Alces alces*).

Hypersexuality manifests itself in a more extreme way in domesticated ungulates. Many of them reach sexual maturity *at the age of 6 months, and may be on heat during the whole* year. The intensified production of sex hormones is more striking here than in captive wild animals. Captivity, however, is to some extent the preliminary step to domestication.

Apart from this hypersexuality conditioned by captivity, there exists an analogous biological phenomenon in those free-living animals for which it is necessary that they produce in a very short time the greatest possible number of young. All conceivable degrees of acceleration, that is to say of overlapping in the different phases of development are possible. Normally there occurs the following order of succession: Anœstral state, œstrus, copulation, pregnancy, parturition and lactation.

Many animals have their new œstrus after the end of lactation, and then another reproduction cycle begins, e.g., in the apes, the bison, etc.

In some cases, for example, in the roe deer, which has a longer pregnancy but a much shorter span of life than man, the animal may come on heat during the period of lactation, so that lactation and gravidity overlap. A prolongation of gravidity is the consequence of this in many species, e.g., in the mouse, as has been shown by Bloch (1948); but in spite of this prolongation, the result as a whole is an acceleration of reproduction.

I believe that the proved prolongation of gestation in the nursing mouse, which Bloch found to be from 21 to 37 days,

occurs in many other mammals too. Breeders of nutria, for example, found the normal pregnancy about 100 days, but for nursing females 130 days. It is quite possible that the extreme divergencies in the data about pregnancy in camelids, which are kept in every zoo, may be traced back to the fact that they include partly nursing and partly non-nursing females. According to data found in literature, the gestation period of camelids varies from $5\frac{2}{3}$ to $14\frac{2}{3}$ months.

The succession of the different phases from one œstrus cycle to the other can be telescoped even more, œstrus [illegible] very beginning, [illegible] ons). But even [illegible] advanced, and we get preparturient œstrus as I have described it in the hare (1948). A highly gravid female (*Lepus europaeus Pallas*) may enter on heat and be served again 3 or 4 days before a parturition and has, therefore, two litters within 38 or 39 days without a male being present. The gestation period is 42 days.

Details of this strange overlapping of two pregnancies are not examined yet, owing to the fact that the hare is rather difficult to keep and may be induced to breed only in so-called adjoining and symmetrical cages, the one a mirror reflection of the other, separated by a sliding panel (Hediger, 1950).

It is to be expected that this kind of super-fœtation is not limited to hares, but occurs in other mammals too. It has even been observed as a great rarity in men too (G. Haselhorst and M. Watzka, 1950). Super-fœtation is quite likely according to A. Jacobi (1938) in the sea otter (*Latax lutris*), and according to K. M. Schneider (1939) in the two-toed sloth (*Choloepus didactylus*). J. Eibl-Eibesfeldt (1950) has often observed that the females of the house mouse (*Mus musculus*), are covered one day before bringing forth a litter.

The occurrence of super-fœtation as a normal appearance in the reproduction behaviour of hare—described even by Herodotus [1]—shows that among the many thousand species of wild animals there are situations completely unknown to the few laboratory animals, though M. Klein and G. Mayer (1948) have succeeded in producing super-fœtation in the rabbit experimentally—at least in the first phases.

Different authors have proved for the horse that it ovulates

not only towards the end of pregnancy, but regularly during the whole luteal phase. It seems that in this respect too, *there are all transitional phases.*

The rabbit is the outstanding example of provoked ovulation. In the ferret, the mink, the cat and some other species, provoked ovulation is known. But based on experience in zoological gardens, it seems highly probable that this occurrence has a much wider distribution than was believed earlier. There is probably no sharp limit between the provoked and the spontaneous type of ovulation. I am convinced that provoked ovulation occurs in the llama, the zebra, especially after a long separation of the two sexes, and Stieve (1950), believes this occurs in man.

Summary

(1) The behaviour pattern of reproduction as controlled by sex hormones shows a much greater variety in wild animals than in the comparatively few laboratory animals investigated so far.

(2) Captivity has a sterilizing effect on some species, a stimulating or " hypersexualizing " influence on others.

(3) Certain environmental factors, e.g., light and food, may have a contradictory effect in different animal species in regard to reproduction.

(4) The normal succession of the individual phases—œstrus, copulation, parturition, lactation, and again œstrus—exhibits varying degrees of concentration or overlapping in wild animals. An extreme case is the hare, in which two gestation periods may overlap, such superfœtation being a frequent and normal phenomenon.

(5) The beginning of lactation may in certain animals (camels, wild boar) precede parturition and, even more so, the ejection of the placenta. In most wild animals lactation may be arrested at any time and without disturbing sequelæ by elimination of the sucking stimulus.

(6) The rôle of the so-called internal secretions, which regulate reproduction is, from the zoo biologist's point of view, seen to depend to a surprising degree on *external* factors.

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DISCUSSION

ZUCKERMAN. Many years ago I analysed the records of births in the London Zoo for the years 1828 to 1937, and I can confirm what you say about the polar bear. To the best of my knowledge, the period within which births have occurred over a century has not exceeded four weeks or so. In some species the records suggested a change in the period of the breeding season, but I do not recollect that any species which in its natural environment was a seasonal breeder became a continuous breeder, and ceased showing any seasonal variation in births. The trans-equatorial shift in breeding season is well known.

BROWNE: Is the normal breeding season of the polar bear that you observed the same as exhibited normally in the wild state?

HEDIGER: As far as I know, it is also in nature.

BROWNE: Professor Zuckerman referred to trans-equitorial shifts. Are there polar bears in the Southern Hemisphere, and if so, does their breeding season differ?

HEDIGER: South of the equator there are no breeding polar bears, as far as I know.

ZUCKERMAN: A few animals when shifted across the equator apparently do continue, for a time at any rate, to breed in the same months they do in their natural habitat. But usually it is found that as a stock of animals from south of the equator becomes established in captivity here, the breeding season very quickly adjusts itself to the new conditions

HEDIGER: The King penguins from the Antarctic adapt themselves more closely to the new springtime every year.

ZUCKERMAN: I think acclimatization of animals has been less studied in this respect than the acclimatization of plants and trees, where there is precisely the same phenomenon to consider.

coasts there has been an alteration in the breeding behaviour, and copulation occurs in the water and lasts for a very much shorter time. In these parts of the animals' range there is a good deal of human activity, and presumably it is the disturbance that has produced this alteration in their breeding habits.

As regards the superfœtation in the hare, I wonder if that is really a true superfœtation? Is it a true second pregnancy, started before the first parturition, or is it merely a survival of spermatozoa, so that fertilization takes place after the parturition. If that is so, it can scarcely be called a true superfœtation.

ZUCKERMAN: Are you suggesting that the sperm go up one uterine

ceptus and fertilize a further ovulation.

ZUCKERMAN: Then you have the difficulty in explaining how the sperm could remain in the vagina after the expulsion of the uterine contents.

there is only a unilateral pregnancy, and the second horn is free. That suggested to us to produce experimentally a unilateral pregnancy and then have a second insemination.

MATTHEWS: Regarding the gorilla, at the Bristol Zoo we had a gorilla, Alfred, who died a few years ago aged about 20. His testes were certainly in an infantile state, with no spermatogenesis, but he was fully an adult, and he had all his secondary sexual characters. He also

ALLEN: Might I suggest that this throws a light on the work of Mott. Human beings have been shut up in more or less solitary confinement in mental hospitals, and Mott found that there was testicular atrophy

testicles were perfectly normal, but the schizophrenics that Mott worked on were patients who had been in a mental hospital for long periods, and theirs were atrophic. It would be very interesting to know if anyone had ever done any work on people with sentences for life imprisonment, who have never had any sexual outlet at all.

KALMUS. Complete cessation of menstruation in many women in concentration camps, with increases in weight and various other correlated changes, have been observed by the camp doctors. This amenorrhœa subsided a year or so after liberation.

BROWNE: It is not necessary to go to a concentration camp. It is very common in nurses in training in a hospital, and known as "institutional amenorrhœa." There it was not a nutritional problem, but it was possibly a nutritional problem in the concentration camps, as has been suggested

women in regard to menstruation. Menstruation did not completely disappear in all women; it disappeared in some definite types of women. And I think it was not the nutritional factor which was the most important one, but the mental stress. There is a corresponding factor in the male: some of them who came back developed gynæcomastia a few months later, and this disappeared afterwards. This shows that there was a disturbance of the steroid metabolism.

LEWIS In the case of the gorilla, was there much evidence of autoerotic activity or ejaculation?

MATTHEWS Yes, autoerotic activity but not ejaculation.

ZUCKERMAN There have been instances of the same thing in chimpanzees, which in Florida breed very well, but which in the London Zoo have usually failed to breed.

UTERINE DISTENSION, OVARIAN HORMONES AND MATERNAL BEHAVIOUR IN RODENTS

MARC KLEIN

SINCE the year 1930, we have performed different series of investigations on the relations between the pregnant uterus and the ovaries in various species of rodents, with the main result that the maintenance of corpora lutea of pregnancy and inhibition of follicle-ripening are found to be conditioned by the placenta inserted on the uterine wall.

In the course of such experiments performed in the rabbit, we have frequently seen a very queer kind of behaviour in the female, which we reported in a paper on administration of steroid hormones and sexual behaviour, delivered at another Ciba Foundation symposium*.

When the whole pregnant uterus is taken away in a pregnant rabbit towards mid-pregnancy, the ovaries being left *in situ*, the female displays three or four days after the operation, the typical nestling behaviour, i.e., making a nest by plucking hairs and sitting on the nest awaiting litters which, of course, will never come. This paradoxical result points to the idea that the distended uterus with subsequent contractions of the muscle are neither an indispensable nor a unique stimulis of the nestling behaviour and of the onset of maternal behaviour.

In order to elucidate this question, Gaston Mayer and I have performed several series of experiments in the pregnant rat. In a first group we tried to maintain the uterine distension at and after the normal term of parturition. In a second group we tried, on the contrary, to produce a premature depletion of the uterine horns with maintenance of a normal pregnancy. Experiments on the role of uterine distension

*This volume, page 323.

in its action on ovaries and mammary glands had been previously performed by Selye, Desclin, ourselves, Bœ and Bradbury, without clearcut and unequivocal results. We and in addition to vaginal epithelium efully studied the behaviour in each individual animal.

In a first group (Fig. 1A), one pregnant uterine horn is ligatured at the lower end; the second horn is left normal. A partial parturition takes place at the normal term. The

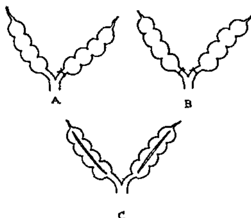


FIG. 1. Diagram of uterus of pregnant rat.
A: one uterine horn ligatured.
B: both horns ligatured.
C: fœtuses removed in mid-pregnancy.

female constructs a normal typical nest, displays normal maternal behaviour, sitting in the nest with the litters, suckling them, seeking and retrieving them. All this despite the complete distension of one uterine horn, containing the overdue, retained and dying fœtuses and placentas. But about five days after parturition, the litter die one after another, and we have not been able to discover the reason for the failure in rearing the newborn. The female is then killed and histological examination shows that the ovarian cycle has restarted, ovulation has taken place, the vaginal epithelium is no longer mucified and the mammary glands

are normally developed and filled with milk. This shows that despite the distension of one uterine horn, the placenta being no longer inserted in the mucosa, the gestatory state of the ovary has disappeared, the cycle has re-started and the maternal behaviour is quite normal at least during a certain number of days.

One may object that a partial depletion of uterine distension, together with a muscular contraction, is quite sufficient to induce the onset of maternal behaviour. Thus we have devised the following second group of experiments (Fig. 1B); a ligature is put on the lower part of each of the two pregnant uterine horns towards mid-pregnancy. A second pregnant rat is put into the same cage as the operated one, this normal female awaiting parturition for the same day as the operated one. As soon as this parturition has taken place, the normal female is taken out of the cage and the operated one will take care of the litters. Despite the complete retention of the pregnancies in the two uterine horns, this female displays a quite normal nestling and maternal behaviour, acting as a foster-mother and rearing up all litters, from which in favourable cases seven or eight may survive. At the fifth day after the term of the parturition which could not take place, the female is killed in order to proceed to the histological examination: the fœtuses are dead, the placenta are no longer inserted on the uterine wall, the corpora lutea of pregnancy are regressing and the follicles are ripening, with subsequent changes in the vaginal epithelium; the ovarian cycle is re-starting, the mammary glands normally developed and filled with milk. It appears from this kind of experiment that despite complete uterine distension and re-starting of the ovarian cycle, without any parturition at the normal term, the female rat displays a normal nestling and maternal behaviour.

We have carried out a third group of experiments in which we produce a partial or a complete depletion of the uterine distension during a normal pregnancy in the following way (Fig. 1C); towards mid-pregnancy, a laparatomy is performed on a pregnant rat and with scissors the uterine muscle is opened, but the membranes of the fœtuses must be carefully avoided. The fœtuses together with their membranes

are extroverted into the peritoneum. Provided the operation has been carefully and successfully performed, the pregnancy

behaviour has appeared whatsoever.

Limited time and space forbid any comment on these experiments, which will probably arise during the discussion. But it seems that the following conclusions may be drawn from the three experiments. Partial or complete distension of the pregnant uterus beyond the normal term of parturition does not prevent nestling behaviour and onset of maternal behaviour. Partial or complete depletion of the uterine tension during pregnancy does not interfere with the normal hormonal conditions of pregnancy, and no nestling behaviour appears. Thus it may be inferred that neither uterine distension nor depletion, in the experimental conditions of this type of investigation, is an indispensable or a primary factor in the induction of nestling and of maternal behaviour. This behaviour seems to be controlled by the changes which occur in the ovary at the end of the pregnancy, changes which are themselves conditioned by the end of the functional activity and of the insertion of the placenta.

An extensive bibliography will be found in the following paper·
KLEIN M. and MAYER, G (1947). *Arch. Sci. Physiol.*, **1**, **11**

DISCUSSION

HAMMOND. In the experiment where the embryos were retained in one uterine horn after parturition, were the retained embryos dead, while the other ones were living?

KLEIN: Yes You will find this in a very careful study by Finn Bøe which first appeared in 1938, and in two papers that appeared in *Acta Endocrinologica* about three months ago, in which he compared the retained embryos five or six days after parturition with the normally born.

HAMMOND. I can argue that it wasn't the uterine pressure at all. It was the case of the embryo and the placenta being living in the one case and dead in the other.

KLEIN: I don't think so, because very often these retained embryos survive 2, 3 and even 4 days after the normal term. As long as the placenta remains inserted in the uterine wall the fœtuses live, but as soon as all the placentas have lost insertion the fœtuses die.

HAMMOND: The fact of the corpus luteum going on suggests that there are secretions coming from the placenta?

KLEIN: Yes.

BEACH: How does this fit in with evidence showing that the pseudopregnant bitch will rear foster young? What is the condition of the uterus in the pseudopregnant dog?

KLEIN: I have never studied the bitch myself. Perhaps Professor Hammond could answer.

HAMMOND: The uterine mucosa of the pseudopregnant bitch is in the glandular phase like that of the rabbit. Pseudopregnant rabbits will frequently make a nest, and this is associated with the dying away of the corpus luteum. I have done some experiments by removing the whole of the uterus and cervix from a pseudopregnant rabbit (*Amer. J. Physiol.*, 1933, 103, 600), and then instead of the corpus luteum dying away at 16 days and a nest being formed, they die away at 24 to 29 days and the nest is formed. It just prolongs pseudopregnancy. Whereas in the ferret and in the bitch pseudopregnancy is maintained for the whole length of the normal pregnancy, in the rabbit it extends only half that time. But the maternal behaviour, in the way of nest-making, comes with the dying away of the corpora lutea. That is, in my opinion, the essential thing.

KLEIN: There is a common factor in all experiments on pregnant rabbits or pregnant rats, the dying away of the corpus luteum.

HARRIS. If you prolong the life of the corpus luteum in pregnancy, is nesting delayed then?

KLEIN: Yes.

HAMMOND. If you inject luteinizing hormone towards the end of the pregnancy in the rabbit, the embryos are retained for about 40 days instead of the normal 32. And under those conditions the nesting begins, not at 40 days, but at about 36 days, when the embryo dies. The embryo in the rabbit won't live longer than about 34–35 days, though you prolong the pregnancy.

KLEIN: If you give small amounts of œstrogens towards the end of the pregnancy in the rabbit, you can prolong the corpora lutea as long as you like, 100 days if you like. Then the fœtuses are retained. They are dead, it is true, but the corpora lutea are maintained. As soon as you stop the œstrogens, the corpora lutea disappear, and immediately the nesting behaviour starts, and a very delayed parturition takes place.

ZUCKERMAN: The conclusion you draw from this is that maternal behaviour is not dependent upon impulses set up in the uterus?

KLEIN: Yes.

THE EFFECT OF DOMESTICATION ON THE STEROIDS OF ANIMALS AND MAN*

CURT P. RICHTER

In my contribution to this meeting I plan to discuss the effects that domestication has produced on the Norway rat, particularly on its adrenal glands; and to bring to your attention the possibility that civilization may have had similar effects on man; and that these effects may help to explain the present-day high incidence of the diseases that are so remarkably arrested by treatment with cortisone and ACTH.

Much of what follows concerns the wild Norway rat, so it may be fitting at this point to explain how I happened to become interested in this hitherto much neglected animal. During World War II our Office for Scientific Research and Development assigned to me the job of developing a poison and poisoning methods that could be put into quick use in case the Axis Powers started germ warfare with rat-borne diseases (Richter, 1945). In this way I made the acquaintance of the wild Norway rat, an animal that previously I had encountered only on a few occasions in the streets and alleys of Baltimore Since then many thousands of them have been brought into my laboratory for study and I have been directly or indirectly responsible for killing many more. I should like to say that I have come to have a very high regard for this animal's wiliness, persistence, and ingenuity.

Darwin (1868), the great pioneer in the study of the effects of domestication, pointed out that animals have been domesticated for a variety of purposes: as means of transportation; as sources of food or clothing; as aids in hunting; or as pets. Another purpose for domesticating animals may now be added to this list: scientific research. The Norway rat may be considered to be the first animal to have become domesticated purely for scientific purposes. How, when, and by whom it was first captured and brought into the laboratory and domesticated has not been established. It is not known whether the first Norways to be brought into the laboratory were wild albino or hooded rats, or whether albinos first appeared as mutations from the regular brown or grey Norways that had been bred in captivity. The earliest report that we have been able to find of its use in the laboratory for a scientific study appeared in 1856 in a paper by a Frenchman, Philipeaux, who curiously used albino and hooded Norways for a study of the effects of adrenalectomy. Norways may have been used sometime before then, but not so long before, since the wild Norway did not come to Europe until shortly before 1800. In the 100 years that have elapsed since Philipeaux' report, the Norway rat has become the most widely used animal in laboratories all over the world. At the same time its free wild ancestor has spread equally widely over the world, on foot and on ships and all other kinds of vehicles to cities, towns and farms.

Compared with the animals that Darwin had available for his studies on domestication, the Norway rat offers much greater opportunities. The ancestors of Darwin's domesticated animals had long since departed from the world scene, leaving records only in paleontological specimens, drawings, and writings—and then only of the skeleton and furry covering. He knew little or nothing about their 'soft' parts—their organs, glands, blood, nor about their physiology or behaviour. Furthermore, not very much was then known about the domesticated animals themselves beyond their skeletal structure, length and kind of hair, and other external features.

In marked contrast, more is known today about the domesticated Norway rat than about any other animal, with

the possible exception of man, since it has been so widely studied in such diverse fields as medicine, physiology, bacteriology, anatomy, and psychology. In addition, its ancestors are available in large numbers within easy access of almost any laboratory, and simple methods have been developed for trapping and handling them without danger of being bitten (Emlen, 1944; Richter and Emlen, 1945). Direct comparisons thus can be made between the wild and domesticated forms, not only of their 'hard' parts, the bones and teeth, but also of their 'soft' parts, such as glands and muscles—and also of their physiology and behaviour.

The wild Norway rat is fierce and aggressive, attacks at the least provocation, is highly suspicious of everything in its environment, ever on the alert for attacks from its many enemies: other rats, dogs, cats, owls, snakes, and man. It must always be ready to defend itself or to escape. It has to forage for its food, fight for its mates, provide its own shelter. In short, it lives under almost constant stress. In captivity it rarely if ever gets over its great suspiciousness, always remaining on the alert for the least change in its surroundings. It breeds very poorly and makes use of any opportunity to escape.

In striking contrast the domesticated Norway rat is tame and gentle and will not bite unless actually injured. It lives in the protected, controlled environment of the laboratory cage, with food and water constantly available, at temperatures which vary through only a small range, with mates generally accessible, and with no natural enemies to threaten its existence. Animals from our stock colony make little effort to escape and can actually be transported in open-topped cages. Obviously, such animals experience stress very rarely, if at all.

The domesticated Norways show all the characteristics of other domesticated animals: high fertility, marked variability, many mutations. Over twenty different strains are known today (Castle, 1947).

Of prime importance for the present purpose is the fact that the Norway rat does not cross with other rats, not even with the wild Alexandrine rat, which in appearance closely resembles the wild Norway. The Alexandrine rat, next to

the Norway, is the most common rat in the world. In many places these two species of rats inhabit the same dwellings, but they have never been known to cross. The wild Norway shows a great homogeneity in many different ways. Obviously if it could be crossed with any other rat it would not be of much use for a study of the effects of domestication.

For comparisons which will be made later with man, it must be mentioned that the Norway rat and man have for countless generations lived in the same parts of the globe, eaten the same foods and shared the same shelters. Furthermore, they have a world-wide population of about the same size and enjoy about the same type of community life.

Now we may proceed with the examination of the anatomical, physiological, and behavioural differences that exist between the two strains.

Comparisons of the organ weights of the two strains shows that some organs have become smaller during the process of domestication; others larger; and still others develop earlier in the domesticated animals. Table I summarizes the results. It is of particular interest that in the domesticated animals, the adrenal glands are smaller, the pituitary is larger, and the gonads and secondary sex organs develop earlier.

Table I

CHANGES PRODUCED BY DOMESTICATION (RAT)

WEIGHT OF ORGANS
(on basis of body weight)

Decreased	Increased	Showing earlier development
Adrenals	Hypophysis	Ovaries
Preputials	Thymus	Uterus
		Testes
		Seminal vesicles
Liver		Prostate
Spleen		
Heart		
Kidneys		
Brain		
Thyroid (?)		
Pancreas (?)		

Comparisons of the physiology of the two strains have revealed a number of differences. The domesticated rats have a much lower resistance to thiourea poisoning than do wild rats (Dieke and Richter, 1945). Many domesticated rats show "running" fits in response to high frequency sounds, whereas it has not been possible to induce them in normal wild rats (Griffiths, 1944). Similarly, domesticated rats kept on magnesium deficient diets develop audiogenic fits and die within 5–15 days; on these diets wild rats have a few fits, but quickly become insensitive, and never succumb to the dietary deficiency (Griffiths, 1947).

Comparisons of the behaviour of the two strains also have revealed a number of differences. One of these came out in a "fighting chamber" that was used to study the reactions of two rats to each other when they are both being subjected to electric shocks. The chamber consists of a wooden box 9 in. × 16 in. × 9 in. with a glass front and a floor made of parallel iron rods, which are alternately wired to the opposite poles of an induction coil. When two domesticated rats are placed in this chamber and are shocked, they leap into the air and run around, obviously trying to escape from the shocks, but they pay a minimum of attention to each other. Wild rats respond in a very different way. Usually at the first shock, they turn on each other and start fighting, each one apparently holding the other responsible for the inflicted pain. An occasional shock is sufficient to keep them fighting almost indefinitely. They bite each other severely, and sometimes one is killed. Another behaviour difference came out in dietary self-selection experiments, in which the rats were allowed to select their own diet from an assortment of 15 purified substances, all offered in separate containers—one fat, one protein, one carbohydrate, 5 minerals and 7 vitamins. From this assortment most domesticated rats make selections that result in normal growth and development. In marked contrast wild rats, apparently owing to their high degree of suspiciousness, will not take any of the substances except the fat and so die in a fairly short time (Richter, 1946). Apparently they would rather die than taste any of the unknown foods. For much the same reason, wild rats are much more difficult to poison.

Other differences have been observed, but I shall limit my discussion to differences concerned with the anatomy and function of the adrenals. Comparisons will be made between the domesticated rats from our colony that has been in existence for about 29 years, and recently trapped wild rats that have been kept in the laboratory for several weeks or months. The colony of domesticated rats started with albinos from the Wistar Institute; and a few years later, black, tan and hooded rats from the colony of Dr. E. V. McCollum were added. Since then no rats from any other strains have been introduced. At present the colony contains an assortment of rats with all kinds of haircoats ranging from solid white to solid black or buff, and containing all variations of hooded types.

The adrenals have undergone marked anatomical and functional changes during the process of domestication. (Watson, 1907; Hatai, 1914; Donaldson, 1928; Rogers and Richter, 1948). They have lost weight as was indicated in Table I. The photographs in Fig. 1 of midline sections from a domesticated and a wild male rat of equal weights (390 grams) illustrate the degree of atrophy that has occurred. The two males were killed under as nearly the same conditions as possible to avoid any effects that might be produced in wild rats by captivity. Both rats were killed by shooting—the wild rat in its native habitat on a farm; the domesticated rat in its cage in the laboratory. The adrenals were removed within seconds after the rats were shot. The individual adrenal glands from the domesticated and the wild rat weighed 18.1 mg. and 93.2 mg. respectively.

In general the adrenals weigh one third to one quarter as much in the domesticated as in the wild rats. Single adrenals from female wild rats have weighed as much as 170–200 mg; from male wild rats, 120 to 140 mg. The differences are localized to a great extent in the cortex (Donaldson, 1928; Rogers and Richter, 1948).

Histological and histochemical examinations of the glands were made in my laboratory by David Mosier. It must be pointed out at once that the wild rat adrenals did not have any pathology that might account for their much greater size and weight. A study of H & E stained sections showed

A.



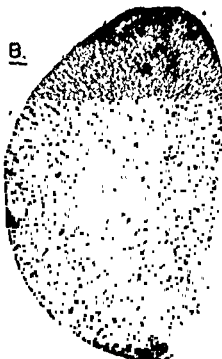


FIG. 1. Photograph of H & E stained sections of the adrenal glands of a domesticated (A) and a wild (B) rat. Both rats weighed 390 grams. Magnification for both sections 50×.

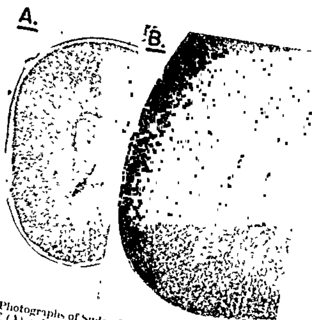


FIG. 2. Photographs of Sudan IV stained sections of the adrenal glands of (A) a domesticated rat (2 per cent salt diet) and (B) a wild rat (25 per cent salt diet). Both had been on the salt diets for 70 days.

that the capsule is wider in the domesticated animals (about 2X) and more cellular. The glomerulosa layer has the same width in both forms but the cells are smaller in the domesticated rats. The fasciculata zone is narrower and has smaller capillaries. The reticular zone is narrower and has fewer capillaries. In the wild rat the reticular group has cell cords that are much more linearly arranged and that tend to follow the lines of the fasciculata. The medullas have about the same sized cells.

Histochemical studies made by Mosier showed that the adrenals of domesticated rats contain a much smaller amount of hormone than do those of wild rats. For these studies, the wild rats were shot in their native habitat and their adrenals were removed within a few seconds after death. The adrenal sections were stained with Sudan IV for lipid content. The glomerulosa layer in the domesticated rat has a sparse distribution of lipid, whereas in the wild rat it has a solid and heavy distribution. The domesticated rat has a well-defined transition zone between the glomerulosa and fasciculata layers; the wild rat has none at all or at most only a very faint one. The fasciculata layer of the domesticated rat also shows a much sparser distribution of lipids. In general, in both strains the reticular zone shows most lipid near the medulla. In some instances, however, the wild rat may show a heavy distribution throughout the entire zone. The smaller lipid content of the adrenals of the domesticated rat indicates a lowered functional activity or hormone content. This agrees with the finding of Nichols (1950) that the adrenals of domesticated rats contain less cholesterol and with our finding that they contain less ascorbic acid (unpublished).

A few differences in adrenal physiology may be cited now. Adrenalectomized domesticated rats can readily be kept alive indefinitely on salt alone, when it is given either in the food or drinking water, but adrenalectomized wild rats cannot be kept alive by salt when it is given in any form or amount (Richter, Rogers and Hall, 1950). Furthermore, we have not succeeded in keeping adrenalectomized wild rats alive with any consistency even with large doses of DCA. Ordinary disturbances of the laboratory or experimentally induced

stress (fighting with other rats in the chamber mentioned above) bring on adrenal crises and sudden death (Covian, 1949).

A second difference between the adrenal physiology of the two strains is concerned with the use of water and salt. A fuller account of these experiments and of their background will be given elsewhere (Richter and Mosier, unpublished). It was found that salt added to the diet produces a greater increase in thirst in the wild than in the domesticated rats, apparently owing to the greater activity of the glomerulosa layer in the wild rat's adrenal, as will be seen below. In one set of experiments, equal numbers of wild and domesticated rats were placed on diets containing salt in various concentrations from 2 to 70 per cent. During an initial period of 10 days, 12 groups of rats, 3 in each group, received our regular stock diet*. Subsequently one group received a low salt diet†, while eleven groups received the low salt diet plus salt in concentrations of 2, 4, 6, 8, 10, 15, 20, 25, 35, 50 and 70 per cent respectively. Much to our surprise, the rats of both strains freely ate all diets with salt concentrations up to and including 35 per cent. Furthermore they all either maintained their weights or actually gained weight on food with these concentrations of salt. Of special interest here is that on all concentrations of salt up to 20 per cent, the wild rats drank more water as measured in cubic centimetres per square metre body surface than did the domesticated rats.

The water intake of the wild rats may reflect a greater activity of the glomerulosa layer of the adrenals, which secretes deoxycorticosterone or a closely related substance for regulating salt metabolism. The evidence for this statement comes from the observations of Ragan *et al.* (1940), and of Rice and Richter (1943), that in normal rats DCA increases water intake in direct proportion to the intake of salt from the diet.

Histochemical studies of the adrenal glands from these rats, which for 70–90 days had received the various concentrations

*This diet contained graham flour 72.5 per cent, casein 10.0 per cent, butter 5.0 per cent, skim milk powder 10.0 per cent, calcium carbonate 1.5 per cent and sodium chloride 1.0 per cent.

†Same diet made without the salt and with saltless butter.

of salt, brought out striking differences between the two strains. The sections were stained with Sudan IV. In the domesticated rats the glomerulosa layer was cleared of lipid even in the rats on the 2 and 4 per cent concentrations of salt, whereas in the wild rats it showed no signs of being cleared at all, even in those on the 20 and 25 per cent concentrations. Fig. 2 shows photographs of Sudan IV stained sections of the adrenals from a domesticated rat that had received the 2 per cent salt diet for 70 days and from a wild rat that had received the 25 per cent diet for the same length of time. The glomerulosa layer of the adrenal from the domesticated rat contains little or no lipid whereas in the wild rat adrenal it contains so much lipid that the limits between it and the fasciculata layer cannot be detected.

A third difference in adrenal physiology between the two strains concerns the factors involved in the production of spontaneous gross bodily activity. These differences were brought out in experiments on the effects of gonadectomy on voluntary running activity. A previous paper describes the cages used to measure activity (Richter and Wang, 1926). They consist of a small living compartment and a revolving drum, both made of metal. The living compartment contains a food cup and a water bottle. A hole in a metal partition separating the cage and the drum permits the rat to move freely from one to the other. A cyclometer registers the number of revolutions of the drum. Fig. 3 shows a typical record of the running activity of a female domesticated rat. The ordinates represent the daily activity in number of revolutions of the drum; the abscissæ, age in days. This rat was placed in the activity cage at an age of 52 days. It was not very active during the first few days, but then rapidly became more and more active and reached a fairly constant high level after about 25 days. It will be noted that the activity reached a peak every 4–5 days. From the work of Wang (1923) it is known that these peaks of running activity coincide with œstrus. Wang showed that gonadectomy performed on an adult rat of either sex produces a profound and permanent depression in running activity as well as in sex behaviour. Fig. 4 gives a typical record. This rat was placed in an activity cage at an age of 97 days

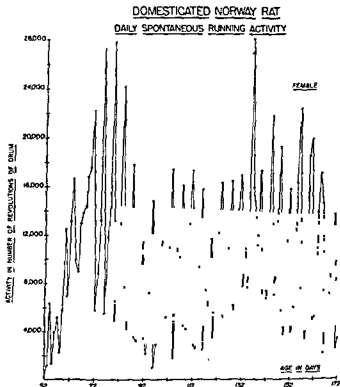


FIG. 8 Typical record of daily spontaneous running activity of a female domesticated rat. Ordinates give activity in number of revolutions of drum; abscissae age in days

and spayed at an age of 121 days. Preoperatively its activity averaged about 12,000 revolutions per day. It showed the 4–5 day cycles. After spaying it immediately became very much less active. For a few weeks it still showed a small amount of activity and then became almost totally inactive. The 4-5 day cyclical changes in activity were completely absent.

In striking contrast wild Norway rats, whether trapped in the streets or born in the laboratory, react very differently to gonadectomy (Richter and Uhlenhuth, unpublished). They remain quite as active as before. Fig. 5 summarizes the observations made on the effects produced by gonadectomy

in domesticated and wild Norway rats. At the left it gives the average daily running activity in 10-day periods for 6 gonadectomized domesticated rats and 10 controls. It shows the great decrease in activity that is produced by gonadectomy in this strain. At the right it shows the same data for 6 gonadectomized wild rats and 7 controls. It will be seen that the gonadectomized wild rats were quite as active as the

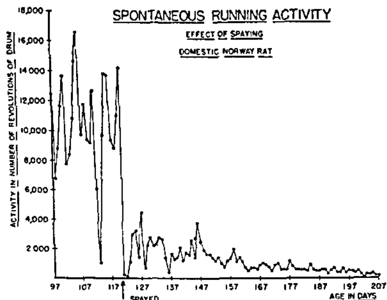


FIG. 4 Typical record showing effect of gonadectomy on daily spontaneous running activity of female domesticated Norway rat.

controls. The females gave essentially the same results. We have not as yet been able to work out any technique for observing the sex behaviour of wild rats, so we do not know whether after gonadectomy they still show any sex interest or behaviour.

To determine whether wild animals of other species likewise remain active after gonadectomy, parallel studies were made on wild cotton rats and wild Alexandrine rats. These two animals were chosen for this study because they are both

... the same size as the Norway

...

and little or no effect on the cotton rats (Uhlenhuth and Richter, unpublished). Thus, it would appear that the spontaneous activity of wild animals in general may be unaffected by gonadectomy. It was likewise not possible to test these gonadectomized wild cotton and Alexandrine rats for sex behaviour.

It occurred to us that the small adrenals of the domesticated rats and their lowered functional activity might in

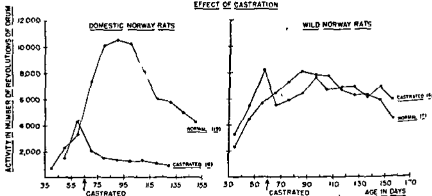


FIG 5. Graphs showing comparison of effects of gonadectomy on spontaneous running activity of domesticated and wild Norway rats.

some way account for their low level of running activity after gonadectomy. To test this idea, gonadectomized domesticated rats were treated with lipo-adrenal extract, DCA, or cortisone* to determine what effect these hormones might have on running activity after gonadectomy. Records were kept also of the effects produced on the vaginal smears.

*The Upjohn Company, Kalamazoo, Michigan, furnished the lipo-adrenal extract; Ciba Pharmaceutical Products, Inc, Summit, New Jersey, the DCA (Percorten); and Merck and Company, Inc., Rahway, New Jersey, the Cortisone (Cortin).

Treatment was started immediately after removal of the ovaries and continued for 40–60 days, at the end of which time the rats were sacrificed and autopsied. The uteri, adrenals and other organs were weighed and preserved for histological study. These experiments are still in progress so the results are not yet complete, especially since no observations have been made on the sex behaviour of these animals before spaying and after the start of the treatment with the adrenal preparations.

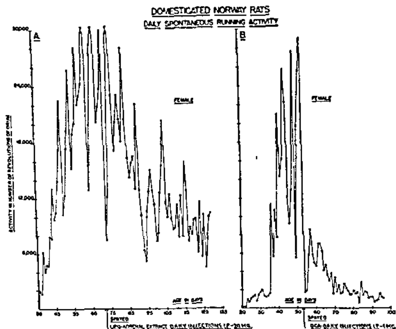


FIG. 6 Graphs showing effect of daily intraperitoneal injections of (A) lipo-adrenal extract, and (B) DCA on the daily spontaneous activity of spayed female domesticated rats.

Fig. 6A gives a typical record for a spayed rat that received daily intraperitoneal injections of 20 mg. of lipo-adrenal extract. This animal was spayed at 73 days of age and the injections were started on the same day. The extract had a

remarkable effect. For the first 20 days the rat remained quite as active as it had been before spaying. After that the activity decreased slowly but even after 50 days it still was far above the level of untreated gonadectomized animals. That these rats treated with lipo-adrenal extract were so active is especially remarkable in view of the large amounts of unabsorbed solvent oil that remained in their abdominal cavities and produced a readily noticeable bulge in their abdominal walls. In more recent experiments we have [illegible] renal [illegible] ay it [illegible] much longer time.

Fig. 6B gives a typical record of one of the spayed rats that received daily intraperitoneal injections of 1 mg. of DCA. The injections produced only a small and transitory effect on running activity. After 40 days the rat was just as inactive as the untreated controls.

Fig. 7A gives a typical record for a rat with a 25 mg. DCA pellet implanted in the muscles between the shoulder blades immediately after ovariectomy. This treatment maintained the activity at a fairly high level for the first 20 days after spaying, but subsequently it lost all of its effect. These DCA-treated rats showed only a very slight effect on the uterus and vaginal smears.

Fig. 7B gives a typical record for one of the rats that received cortisone in its food—0.33 mg. per gram of food or a total of about 5 mg. per day. This animal was spayed when 81 days old and immediately placed on the cortisone diet, which maintained its running activity at approximately the preoperative level for over 20 days. After that the activity fell off but still remained well above the level of the untreated controls. Particularly noteworthy in Fig. 7B is the presence of four definite 4–5 day cycles of running activity during the first 28 days on the cortisone diet. Incidentally this observation brings further evidence in support of the view that the 4–5 day cycles have their origin in the adrenals rather than in the ovaries (*del Castillo and Calatroni, 1930; Zuckerman, 1938*). It will be of interest to see whether castrated males treated with cortisone also show 4–5 day cycles of activity.

These experiments on gross bodily activity have demonstrated a marked difference between wild and domesticated rats that can be explained at least in large part by a deficiency of the adrenal glands of the domesticated rats. The results indicate that the adrenals of domesticated rats must be deficient in cortisone and DCA, and that they may lack the

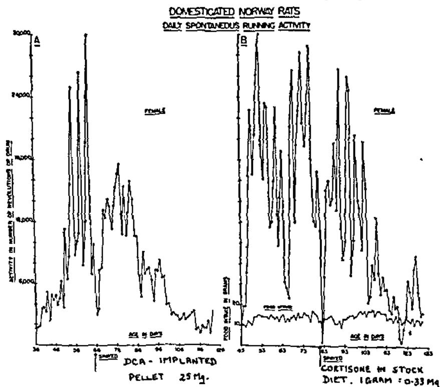


FIG. 7. Graphs showing effect of (A) implanted DCA pellet, and (B) cortisone in food on daily spontaneous activity of spayed domesticated rats.

œstrogens and androgens shown by Engelhart (1930) and otl

ph

an

my mind the changes can be explained in large part on the basis of the operation of selection—not selection in the natural or free state, but selection in an artificial or controlled environment. In the wild state the fiercest, wildest, boldest rats, the "fittest" for that type of environment, undoubtedly have the best chances of surviving; in controlled conditions of the laboratory, on the contrary, the animals that are the tamest, gentlest, least apt to escape or give trouble, the "fittest" for that type of environment, will have the best chances of surviving. The most critical stages of this selection process are undoubtedly the times of mating and nursing. Wild rats are so suspicious of the laboratory environment that except in rare instances they will not mate in captivity. Thus it will be the tamest of them that propagate their kind. An even more severe screening process occurs during the nursing period, when at the least provocation, a wild mother will kill her entire litter. Here again it is only the offspring of the least apprehensive that have a chance to survive in the laboratory.

The observations reported here have shown that marked differences exist between the domesticated Norway rats of our colony and recently trapped wild rats. Similar comparisons have not as yet been made with domesticated rats from other colonies; so it is not known just how representative these data are for all domesticated rats. From data published from other laboratories it is known that the adrenal weights from many different strains have essentially the same values as were found for our rats. Data that have been published for other organs and glands also are similar, so it is likely that our rats are fairly typical for other domesticated strains.

The results of these studies have given us a glimpse into the realm of changes that may occur in individuals of a large animal population when the animals are taken out of their free environment and placed in a controlled environment.

It may be asked now whether the knowledge obtained from this glimpse can be of any help in throwing light on what has happened or may still be happening to man during the process of his becoming domesticated or civilized. Or in other words, is man more like the wild or the domesticated

rat ? Obviously it is not possible to give a simple answer to this question.

It seems likely that man, like the wild Norway, originally lived in an environment in which he had to search for his food, provide his own shelter, fight for his mates—an environment in short in which his fitness, hence his survival, was measured by his physical activity, aggressiveness, and ability to withstand violent change. But with the growth of communities and the consequent increase in daily peace and security for the individual, a new environment developed and may still be developing, in which man is protected from enemies—at least internal ones—and his food, shelter, and livelihood are guaranteed. Man thus may have worked out a controlled environment for himself in which a transformation occurred, somewhat like that undergone by the Noway rat in its adaptation to colony life in the laboratory, but an even more gradual one in which the qualities of physical strength, fierceness and aggresiveness would no longer be at such a premium. Hence would result the increase and perhaps even the predominance of the progeny of the weaker, milder, " better adjusted " individuals.

Here, just as with the domestication of the rat, a selection process, the selection of the " fittest " for this type of environment, would probably play the most important part.

In the light of such a development, changes in the adrenals and other organs such as we have demonstrated in the rats, might be expected to have occurred in man during his long ages of " domestication ". Unfortunately at present we lack data on the endocrine organs of aboriginal man to compare with those of our contemporaries.

Such an assumption leads directly to consideration of the possibility that the widespread incidence of those diseases that are so remarkably arrested or altered by the administration of ACTH or cortisone—as seen in the 10–12 million persons suffering today from rheumatoid arthritis, asthma, and other hypersensitivity diseases, and possibly some mental diseases—may represent the end results of the process of becoming civilized. The fact that these persons are helped by cortisone and ACTH only so long as the treatment is continued indicates that the adrenals have

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[illegible] *J. Lab. Clin. Med.* 7, 289.
[illegible] *nology*, 42, 46.
[illegible]

DISCUSSION

Zuckerman: I am much interested in the observation that spayed animals exhibit a 4-day cycle in running activity when maintained on constant amounts of cortical hormone. The observation parallels an earlier finding of ours, that if spayed rats are kept on very low and sub-threshold constant doses of œstrogen, 4-day cycles also occur. We tried to discover the source of the œstrogen, which we assumed was being produced endogenously and cyclically to supplement the injected hormone, and came to the conclusion that it was the adrenal cortex. Adrenals removed during œstrus from spayed rats maintained on a constant low level of œstrogen were larger than the adrenals at other times of the cycle.

Could Dr. Richter tell us whether the wild rat's adrenal can be made to hypertrophy still further?

Richter: I can't answer that now. We have experiments in progress to determine whether after removing one adrenal the other adrenal becomes larger as it does in the domesticated rat

Zuckerman. Have you a "hunch" which way the experiment will go? Do you think that the potential ceiling has been reached by the wild rat's adrenal?

Richter: I haven't any hunch. I couldn't even speculate. But I might just mention that in a few instances we have implanted the adrenals from wild rats to the eyes of domesticated rats, and found that they grow at a very rapid rate.

Zuckerman: Among the changes which you suggested had occurred during the course of domestication of the albino rat are the precocious appearance of the sexual cycle, the precocious development of sex [illegible] in the size of the adrenal cortex [illegible]

Richter: I don't know whether that follows or not. The pituitary is larger in the domesticated than in the wild rat. The pituitary may be trying to buck up the failing target organs, certainly the adrenal. [illegible] that some abnormal one [illegible]

undergone permanent changes and so are no longer able to take over their normal functions.

In the light of such an explanation as to the origin of these diseases, it is possible that we ultimately may find that a high price has been paid in corticoid hormones for the security provided by a controlled environment.

Thus, in summary, the Norway rat, the first animal to be domesticated for purely scientific purposes, has during the process of domestication, while becoming adjusted to living under controlled conditions in the laboratory, undergone marked changes in anatomy, physiology and behaviour. The observations and study of these changes may help us to understand and evaluate what changes civilization has produced in man, particularly the changes that may have occurred in the all-important steroid producing adrenal glands.

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RICHTER: Yes.

PINCUS: May I ask about the transfer of wild young to domestic nurses?

RICHTER: That is something which is in progress now.

PINCUS: I tried that a number of years ago and I know it is feasible. We had wild Norways, the young of which we transferred to domestic animals, and they were raised successfully.

KALMUS: Albino rats are only the most extreme form of coat colour mutants. Keeler has done some work on tameness and colour in various mammals, and he seems to have proved, to his own satisfaction at least, that tameness goes hand in hand with colour dilution and various other colour variants.

I think a very interesting and hopeful situation exists here for the study of evolutionary processes, in that you have available these wild

than was formerly suspected. If you could overcome the technical difficulties with your rats, I think that it would be very worth while trying. Crossing wild rats with rats kept in captivity, you might expect to get in the second generation a quarter wild and three-quarters tame rats.

One further point ought to be made here: that is the delusion that environment can always be neatly separated from genotype. A laboratory milieu is obviously quite different in its impact on tame and on wild rats, and it is difficult to devise an environment which is fairly normal for both types. Also I think that great care should be taken

HEDIGER: I think it might be advisable to distinguish clearly between domestication and tameness. Domestication is a process which extends over many generations, whereas tameness may be acquired by an individual. In animal psychology a tame animal is defined as an animal which has no flight tendencies against man. And it might be very interesting to examine the adrenal glands of tame wild rats.

RICHTER: We have had some success in taming very young wild rats. Even, however, when well trained they remain very nervous, constantly quivering when handled, and ever ready to bite, jump or escape. They retain their large adrenals.

in which we removed one [illegible]

ovaries. Some observations which Mr. Eayrs has made on lactation suggest, again, that when rats are kept in the dark their powers of lactation are affected All this suggests that not just ACTH secretion, but other pituitary activity as well, may be affected under abnormal and possibly stressful environmental conditions.

FOLLEY: Lactation could be affected by ACTH

ZUCKERMAN. Lactation could be, but would the secretion of ACTH be affected by keeping rats in the dark?

PINCUS There is the possibility of differential inhibition. Adrenal steroids will inhibit ACTH. If you are assuming that these animals are hyperadrenal, you have a situation in which they pour out a lot of steroids, and it should inhibit the ACTH if they have these enormous adrenals. Therefore, I suspect that the situation is that there is a greater sensitivity to ACTH in this adrenal tissue, and Dr. Richter's transplant experiment would suggest that. When he transplants the wild adrenal into the eye of the domesticated animal and gets this enormous hypertrophy, that would suggest that relatively small amounts of ACTH would be much more effective in the wild rat adrenal than in the domestic rat. That is a very interesting situation; I know of no parallel.

DEMPSEY: This raises a point that has always interested me a great deal. We tend in all these endocrine phenomena to interpret changes in terms of an increased or a decreased amount of the stimulating hormone and usually ignore possible changes in the sensitivity of the target organ, which might explain the phenomenon just as well.

FOLLEY: How long after gonadectomy did you test the running activity of your gonadectomized rats?

RICHTER· I think the longest was about two months

FOLLEY: Woolley and Little found in their mice that some months after gonadectomy the adrenals begin to hypertrophy. Mammary growth occurs, for instance. I wondered if you had gone on long enough to find a gradual increase in the running activity?

RICHTER: These experiments were only run for two months. But we have run the castrates for years. They never become active again.

BEACH. Do your wild gonadectomized animals that continue to run cycles also show œstrus, physiological or behavioural?

RICHTER: We haven't made any sex tests on them.

ZUCKERMAN: In point of fact you would find that there would be a cyclical alteration in the character of the vaginal smear"

Richter: Yes. Some of our rats showed 4-day cycles, even with getting the cortisone in their food.

Cleghorn. Is the 4-day cycle as marked?

Richter: In one of the charts that I showed there were four very regular cycles during the first 28 days after the rat was spayed and treated with cortisone. After that the activity fell off and any tendency to cyclical variations disappeared.

whether cats can be found wild in Baltimore, they certainly can in this country—and to wild dogs, although no doubt the Australian Government would have something to say if we started to exterminate dingos.

Editor's Note.—At this point a film was shown, prepared by L. V. Domm and D. E. Davis (University of Chicago), on "The sexual behaviour of intersexual and hormonally treated domestic fowl".

MATTHEWS: As regards the effect on human populations there are some interesting data on population cycles in small rodents, for example the field vole. Recent work has shown the possibility that the crash after the huge build-up in population is due to a general adrenal exhaustion, throughout the population.

KLEIN: In the comparison of the organs of the wild rat and the domesticated rat, have you paid any attention to the sensory organs and especially to the retina? I am asking this question because we have ... laboratory a strain which shows the heaviest ... hings have been ... by Bourne and ... ticated rat need

RICHTER I can't answer that. The only observation we made on sensory organs is that there is a reduction in the number of fungiform papillæ on the tongue—about 20 per cent. There is no change in the number of foliates.

HAMMOND: Has the body weight changed?

RICHTER: The body weight has changed a little. There has been no change in the skeletal size at all. The total length with relation to weight has stayed about the same, so externally there is really no change

HAMMOND: Those weights of the organs that you gave, were those actual weights?

RICHTER: No. They are per kilogram of body weight.

HAMMOND· We find in domestication of sheep, etc., that the total body weight goes up, but the various parts and organs go up in proportion to brain weight. In wild animals the brain is proportionately heavier in relation to body size; that is, brain weight is much more constant than the body weight.

... to be reduced in size to about ... adrenal ... iantita- ... extract injected prior to a stress stimulus and the inhibition of the adrenal response to that stimulus Does such a relationship also hold in the wild animal?

RICHTER: That is something that is also in progress now. I have a number of hypophysectomized rats going now. We're letting them run for six months before we autopsy them.

BROWNE: Does the activity of spayed rats treated with œstrogens decline in a similar way to those treated with cortisone or adrenal extracts? In other words, does that decline also occur under œstrogen therapy?

RICHTER: Yes.

increasingly so as the discrepancy between the individual and his fellows increases. These people are, however, frequently well muscularly co-ordinated and can, and frequently do, perhaps as a compensatory mechanism, indulge in athletics in proportion to their size. They can be made to grow by the use of various hormones, including testosterone.

The first case was aged thirteen. We gave him chorionic gonadotrophin for a period of time, and a certain amount of growth did occur. Then we gave him testosterone propionate, 25 mg. three times a week, and there was a sharp increase in his growth curve, which ceased when we stopped the hormone, and began again when we started treatment again. After the dosage was stopped for the second time, the growth continued for a period, unlike the sharp cessation in the first interval. This was accompanied by spontaneous sexual maturity, in spite of treatment with testosterone. Yet although this boy grew and developed sexually, his psychological make-up, shown by his behaviour, and particularly by repeated Rorschach tests, showed absolutely no indications of sexual maturation from the psychological point of view. So here we have an instance where the application of a hormone produces a physiological effect in terms of growth (it relieves his psychological distress in terms of his undersize) but it does not necessarily produce any psychological maturation from the sexual point of view. In other people that is not so; it is often very clear under this treatment that there is maturation both from the psychological and from the physical point of view. But there can be marked discrepancies in this.

Another example shows the sort of psychological distress which may occur in this condition. I don't think this is specific to this condition, but since it shows up so beautifully some of the ways of obtaining information about families, I would like to draw it for you. This was found out by Dr. Doris Menzer, who was a psychiatrist attached to our department some years ago, in a boy who had been brought to see me because he did not grow. He started to draw a number of things, and he drew the family dinner table. He drew himself much smaller than his younger brother and much smaller than his younger sister, who was considerably younger

PART II

PSYCHOLOGICAL AND BEHAVIOURAL REACTIONS CONNECTED WITH PATHOLOGICAL DISTURBANCES OF STEROID HORMONE PRODUCTION

THE INTERPLAY BETWEEN ENDOCRINE DISTURBANCES AND PSYCHOLOGICAL ABERRATIONS

J. S. L. BROWNE

I THINK it is fairly apparent that in the case of human beings it is extraordinarily difficult to assemble an adequate number of patients and to control patients' behaviour, and therefore I have thought it best to discuss a few cases, from a longitudinal point of view, since I regard the internal control as an extremely important feature in the study of biological aspects in man. Also I would like to emphasize, although it is perhaps not strictly speaking part of the subject of this congress, that there is after all an influence in reverse of emotional states upon endocrinological and glandular patterns, and the consequences of this are the same as if these glands were stimulated in other ways. I propose to discuss a number of different glands, and to leave until tomorrow discussion of the effects of injecting adrenocortical extracts, cortisone and ACTH.

First of all there is the problem of the individual who does *not grow*. We see a number of different types of people of this sort, but I propose to discuss one particular group, those *who could be called delayed puberty. These people are short;* they do not grow. They ultimately do mature and grow, but *may not do so until about the age of 16 or 17. The exact* pathogenesis of this condition is not known, but it is not accompanied by *any gross abnormality of the pituitary.* Its psychological effects are very marked indeed, and become

thirty a day, and they were controlled on 2 mg. of œstradiol dipropionate. Dr. Menzer after several interviews persuaded her that she could tell her father without killing him, and she did; and she separated from her husband. The hot flushes were then completely controllable on 1·65 mg. of Premarin per day. This went on for three months. She then came back complaining that the flushes had recurred, and upon enquiry it was found that she had allowed her husband to re-enter the house and had taken up relations with him again. In other words, on a constant dosage of œstrogen, differences in psychological state could and did change the symptoms. It is true that this is only one case and we cannot conclude much from it, but in this case it did seem to me that the level of œstrogen dosage at which hot flushes occurred was different depending upon her psychological state. So there again we have an i [illegible]
the requirements, [illegible]
the organism to a [illegible]
taneously secreted hormone.

Now we come to the problem of adrenal responses, and I want to stick to the spontaneous responses of the adrenal to emotion and not to the influences of the adrenal cortex upon emotion, which we will discuss later. There is in pregnancy a double peak, one at about sixty days and one in the seventh to eighth month, as shown by Dr. Venning. Here we have a situation in a physiological condition where the level of corticoids is not indicated by the ketosteroids, either by the Zimmermann or by the Pincus method. There is no increase of those, and yet there is a very great increase of the corticoids. If we regard that as being a true index, that the adrenal has increased its secretion very markedly, you will observe that the levels reached are quite in keeping with those seen in Cushing's syndrome. One might describe pregnancy as being in certain ways a physiological Cushing's syndrome. [illegible]
ture, the app [illegible]

[illegible]

certain of the psychological changes in pregnancy.

than he was. He drew a book of trains which the father had just broug[illegible] her says, "Here you are," [illegible] !", the younger sister says [illegible] says, "What a nerve!", and he says, "How can you do this to me?" Some of this was said and some was only thought. Now that makes the father look like an insensitive type of person, but it was found in his history that he had been filling the position of a father to his younger brother; he brought him up, and then the younger brother turned out to be no good. He decided when his first son was born that he wasn't going to do it again, and consequently he neglected the child; then he forgot all about it with the younger brother. This was re-adjusted rather simply and the child grew up perfectly satisfactorily without any other treatment. That can, of course, be due to interference with food intake and so forth, so it is to my mind perfectly possible for psychological distresses of ordinary life to occasion failures of growth of this type. We have all heard about hypothalamic amenorrhœa, and it seems to me that other abnormalities such as this, where we cannot detect [illegible]

With regard to the ovary, I would like to point out one particular case which I thought was rather striking. It is extraordinarily difficult, as you well know, to get quantitative measurements of the effects of œstrogens in man. This woman was sent to me because she had hot flushes which had not responded to 1·65 mg. of Premarin. I found that she was a young woman who had been ovariectomized on the demand of her mother. The mother ran screaming down the corridor after this was done, saying that this was a terrible thing to do to a young girl. Her husband had a colostomy, and thinking he had only a certain time to live, had deliberately become promiscuous and unfaithful to her. The mother forbade her to discuss this with her father because he had a coronary and it would kill him, so that there was considerable discobobulation of the family life. This woman's hot flushes were quite obvious and classical; she had about

cause fairly continuous over-production of cortical hormones of this type, and these, at a lower level than that required to produce overt Cushing's syndrome, might easily produce the same effects as the administration of moderate amounts of cortisone, or of ACTH. That may or may not be true, but I think it is worth investigating.

As an illustration of the influence of psychological stress upon adrenal cortical hormone output in the urine, as measured by the Venning method, a normal woman whom Dr. Venning was following put out 40 μg. equivalent of Compound E per day, which is normal. Her sister became very ill, and the next day her corticoids were 300 μg. The woman was perfectly well but was greatly distressed by her sister's [illegible]

after her sister. It seems to me, and it has been shown previously by Dr. Pincus in other ways, that emotional stress can fire the adrenal, and there does not have to be a major physical injury. Dr. Venning found, for example, that in technicians going to bargain sales on Saturday, the adrenal cortical output went up very definitely, so that we don't have to demand that something extraordinary shall happen to the individual for the adrenal to respond. Of course, it doesn't in that instance go up nearly to the level found in a surgical operation, but it can respond. It also seems to me that this is extraordinarily difficult for us to control. If we want to find the response of a group of normal individuals to a certain treatment, we generally bring them into hospital. Now the very fact of bringing them into hospital is in itself [illegible]

duced, in part at least, by certain psychological effects.

I therefore think that in a number of fields there is this interplay between the endocrine system and psychological aspect in both directions. I do not propose to go into the tremendous area of abnormalities like hirsutism, the

I would like to talk today of only one effect of cortisone, because it comes into the problem of psycho-physiological inter-relationship, and that is the effect on appetite. In young children, aged two to four, treated for nephrosis, the effect of cortisone or ACTH, whether or not there is a diuresis, is to produce a ravenous appetite. One of these children—and this was common—ate eight lamb chops and two beef-steaks per day, with all the accompanying potatoes and so forth. When she diuresed she was three years old and weighed 22 lbs. so she certainly did not synthesize most of this into her body protein. Children who are somewhat older, say the rheumatic fever group, also get a marked increase in appetite. In general, adults also get an increased appetite. We have had two instances of diminution of appetite in 150 cases, and those occurred in individuals who were temporarily improved in their condition, but who then became convinced, as in a case of Hodgkin's disease for example, that the disease was not going to go away, and they went into a depression and lost their appetite. This did not always occur when the individual's disease was obviously not going to be much improved; often their appetite still increased.

I have held the view that obesity was very largely psychologically conditioned. I have found in practically every instance of obese children, obese girls particularly, that the patient is the "mother-daughter," and that over-protection or rejection is the usual feature. If you ask the child how old she is, she generally will turn her head towards the mother, who replies, "She is sixteen." I once had a child with both the father and mother there, and the poor child's head was [illegible] I felt that the mechanism for [illegible] d- [illegible] ho found ravenous increases in appetite in animals after injuries to certain parts of the hypothalamus. But seeing these results with cortisone at a dose level which does not produce Cushing's side effects, yet does produce obesity because they eat a great deal, it occurs to me to wonder whether I may not be wrong and the endocrines may have something to do with obesity. There is no reason why, as I shall show in a moment, psychological stresses cannot

the uterus removed and the ovaries left intact. Of course, the ones that had had the castration developed the flushes, etc., which responded to œstrogen; but the women who had not been castrated developed psychological reactions to the interference with their normal life (cessation of their periods, and so on) which included flushings and other anxiety reactions, autonomic disturbances, not attributable, of course, to any gonadectomy One could have easily fallen into error, if one had assumed that when flushings occur they are attributable to the menopause

BROWNE: On the other hand, this case does illustrate that the

agree with you that a great many symptoms of the menopause are, of course, on a psychological basis.

LEWIS: Some women during pregnancy or during the post-partum period develop a psychosis, which is accompanied by hairiness and other signs which rather suggest Cushing's syndrome. Has the endocrine change ever been confirmed by closer physiological examination? I don't know of any instance where it has

BROWNE I don't know. Perhaps Dr. Pincus does.

PINCUS No. But the incidence of psychosis in Cushing's syndrome is reputedly high.

SIMPSON· Five out of nine of Cushing's original cases, in women,

PINCUS: Has the behaviour of women during the period of maximum cortical excretion been defined, analysed?

BROWNE: I assume that the psychological changes occurring during pregnancy have been, but I doubt whether the timing of them has been investigated from the point of view of the hormone assay. I still feel that in doing this one should deal with individual cases, doing close continuous psychological study.

CLEGHORN. This has not been done. Pregnancy has not been thoroughly investigated from the psychiatric point of view.

LEWIS I confirm that.

ZUCKERMAN. But I take it that there is no indication that all psychiatric disturbances which occur during pregnancy are related in some sort of way to a disturbed metabolism of the adrenal?

REISS: I would put it the other way round. The endocrine changes seen in pregnancy can in certain individuals of a certain constitution produce certain psychiatric disturbances.

psychological effects of these abnormalities, and the possibility that in turn the psychological upsets may cause endocrine disturbances. One patient said to me, "When I get emotionally upset, my hair grows much more rapidly, I am sure of that." And I see no reason why that should not be so if the adrenal is producing an abnormal amount of androgens; they could be increased by emotional stress just as easily as can the glucocorticoids.

DISCUSSION

BEACH: Dr. Neal Miller and some co-workers recently showed that rats with hypothalamic lesions show little or no hyperphagia if they are compelled to work for their food. Perhaps the effective lesions damage the "shut-off" mechanism that normally leads to cessation of food intake once hunger has abated.

ALLEN: Turner and Miles have found that there is delayed puberty in intellectual types of children who were made to take examinations. The intellectual pressure seemed to delay their puberty. I wondered whether, when there *is* emotional stress, that delayed puberty can't shade off into a eunuchoidism? I have seen a number of cases—although I am not connected with them specifically—of boys who have had difficulty with their parents, particularly the same type of case as Dr. Browne advanced, who were eunuchoid, and in some cases schizophrenic. I do know a case, which I had the opportunity of observing for about 20 years, where the father was constantly nagging at the boy until the poor boy didn't know what he was doing. He grew up and became schizophrenic, and later on quite definitely eunuchoid. I have seen similar cases in which there seems to be a tremendously strong emotional element This boy was extremely hostile to his father, and it seems as though in suppressing the hostility, he suppressed his whole sexual development. That may be too exaggerated, but that is what to me seemed to be happening.

BROWNE: I am sure that these psychological stresses, as I might call them, will obviously be differentiated in their effects through the whole structure of the body. In other words, one individual may develop eunuchoidism under them and another may not. The nature of the psychological stress may be very similar but its effects will differ probably, dependent upon the individual.

ZUCKERMAN: Did the father to whom Dr Allen referred behave the same way to his other children? Or was his peculiar behaviour inspired by something in the boy?

ALLEN: *Only to the son.* He had a daughter, who was his favourite, and she has grown into an extremely charming and normal girl.

LEWIS: With regard to these flushings where œstrogen administration was effective, I once examined a series of women who had artificial menopause, induced surgically, and another group who had had only

Sections). For some reason it was not recorded, but I would like to quote briefly from my contribution :

"Endocrine manifestations or stigmata may result from

of endocrinological pathology in a psychotic or neuropsychotic, it by no means follows that these are primary. On the other hand, it is undoubtedly true that many patients with *endocrine disorders develop, concomitantly or subsequently*, symptoms of psychoneurosis or psychosis ; and, further, that successful endocrine therapy will often tend to abolish the associated mental abnormalities.

Apart from the well-defined psychoneuroses, the endocrine balance of an individual, either grossly abnormal or merely constitutional and familial, or physiological (puberty, pregnancy or the menopause), has an important influence on the character, behaviour pattern and emotional reactivity. Such

not fail to recognise a close association."

I ventured to describe briefly just two interesting types :

Firstly, the post-menopausal, in which, secondary to

may become aggressively active, enterprising, organising, dominating, and often manifesting considerable ability. Such types include brilliant women business directors, suffragettes and their modern equivalent, the conventional mother-in-law, the wives of henpecked husbands, and, if sufficient feminine characteristics are maintained, the all-powerful Matriarch of G. B. Stern and other novelists or dramatists.

Secondly, a pituitary type of adiposity as typified by the fat girl or woman, with slender limbs, a beautiful and lovable face, good teeth, and a fascinating smile ; wide social leanings, loving and loved, perhaps made fun of but extremely popular, a good dancer in spite of corpulence, very musical and often

BEHAVIOUR PATTERNS AND PSYCHIATRIC DISTURBANCES IN MAJOR ENDOCRINE DISORDERS

S. LEONARD SIMPSON

Summarising at the beginning instead of the end, my clinical impressions as an endocrinologist are that major endocrine disorders are not infrequently associated with psychoses, psychoneuroses or characteristic behaviour patterns (this subsequently being termed for short P.P.P.); that the disturbed hormone secretion or the resulting metabolic change is a direct cause of the P.P.P., e.g., hypersecretion of adrenal corticosteroids, hyperinsulinism, hypoparathyroidism, although pre-existing genetic factors influence the character and intensity of the response. In some cases, the problems and sufferings of the endocrine condition, e.g., extreme hirsutism, are themselves sufficient to produce P.P.P. apart from any direct hormonal stimulus. Fortunately, greater awareness has resulted in there being fewer people with unrecognised endocrine disorders in asylums to-day than there were previously. The treatment of major endocrine disorders does not necessarily reverse associated P.P.P., especially if therapy is belated.

However, I do not believe that endocrine disturbance is a relatively frequent cause of the psychoses or psychoneuroses as they present themselves to the psychiatrist; it is probably a rare cause; further, many psychotic patients develop endocrine stigmata as secondary features of their illness. As to behaviour pattern in children, Lurie, in Ohio, found a 20 per cent incidence of endocrine disturbance in 1,000 children at a child guidance home. As to deliquency: as Consultant Endocrinologist to the Institute for the Scientific Study of Delinquency for some 15 years, I find that endocrine disorders are only a rare cause of childhood delinquency.

In February, 1937, there was a joint discussion at the Royal Society of Medicine (Psychiatric and Medicine

As to acromegaly, I wrote in 1938: "Speech may be sluggish and slow, memory often impaired and general behaviour characterised by apathy and lack of initiative. Depression, irritability, negativism, melancholia, mania and delusional insanity may occur. In the early stages or in relatively mild cases, there may be great alertness, energy and drive." One should endeavour to distinguish initial phases of hyperactivity and later phases of involution or exhaustion, particularly involving the adrenal and thyroid glands. However, adrenal androgenic hyperfunction with extensive hirsutism may occur when there is concomitant thyroid deficiency. I am impressed by the great strength and driving power of some medium giants, and even mild acromegalics who might well be classified within physiological limits.

In a recent biography on a famous economist, the author, without medical knowledge, writes "a fastidious, ailing product of a day school suddenly became by accident of premature growth and a broken voice, the spokesman and leader of his group at Eton." This also brings in the important effect of puberty on intellect, character and physical strength.

In contrast with the initial active phase of hyperpituitrism, we have the apathy, inertia and somnolence of Simmonds' disease or pituitary hypofunction, the lack of initiative, spontaneity and drive. I am sure this is not entirely explained by hypoglycæmia, although this plays a very significant part, but rather as the opposite of gigantism or early acromegaly. Certainly, testosterone therapy alone can make a big difference.

One of my patients with Simmonds' disease, an ideal domesticated wife, lost all interest in her home and family, including her two children, for whom not a remnant of maternal instinct remained. Another charming and smart woman became completely indifferent to her clothes and appearance and even dirty in her habits and hygiene. As have others, I have met with depressive states and delusional insanity in chronic untreated Simmonds' disease.

In Addison's disease, the same apathy and lack of initiative may be met, even with patients whose mineral metabolism has been returned to normal. There is in addition a greater

gifted, a warm quality voice, artistic temperament, romantic nature, with alternating phases of happiness and depression that may recall to mind a lesser grade of the manic-depressive psychosis.

This second description applies to a group of girls I later classified as adipose gynism—a syndrome caused by pituitary hyperactivity (including growth hormone) and some elements of adrenal cortex hyperactivity. The girls are intensified females whereas the males, to whom I applied the term adipose gynandrism, are feminine type males. Apart from delayed puberty in these males, their voice, gestures, gait, emotionalism, exhibitionism, sensitivity, potential outbursts and general behaviour tend to be feminine. All this might, however, undergo appreciable change in the later phase of adolescence, if an intense androgen phase was superimposed, but in other cases the behaviour pattern persisted.

Mellicow and Cahill (1950), in discussing somatosexual disturbance in children, differentiated two types, the corticosexual androgenic syndrome being emotionally well balanced and the corticometabolic Cushing type syndrome being emotionally unbalanced. This rather fits in with Sprague's therapeutic experience (1950), of a boy of 2½ with macrogenitosomia who became wildly excited and maniacal after 50 mg. cortisone were injected daily for some days.

Wilkins' patient (1940), however, a boy of 3½ with an androgenic adrenal sexual precocity and associated adrenal insufficiency, growled and snarled when attempts were made to examine him.

Cushing's syndrome in adults is certainly frequently associated with P.P.P., and these are too common to be regarded as chance associations, apart from the knowledge we have gained as to the effects of cortisone in non-endocrine disease. Depressive states, paranoia, and suicidal tendencies are, in my experience, the more frequent psychotic manifestations. One of my patients committed suicide after she was making considerable physical progress some months after a course of radiotherapy to the pituitary gland. Of two cases recorded as successfully treated by Swan and Stephenson (1935), with radon seeds in the pituitary gland, one later developed delusional insanity.

initiative, felt like someone dying and showed lack of application and concentration."

A woman, castrated in infancy by an operation on an inguinal hernia containing both ovaries strangulated in the inguinal sac, showed none of the above characteristics. She was a charming and successful woman, of many interests and obvious ability and normal sexual activity. Women, ovariectomised in adult life, may show climacteric changes and repeat these again in the fourth or fifth decade.

Having summarised before I started, I would conclude

ceptibly into normality. Therefore, if it is granted that psychoneuroses and behaviour patterns are associated with and may be caused by major endocrine disorders, I would suggest that we develop an awareness of their possible occurrence over a far wider range in association with minor hormonic disturbance or incomplete syndromes. This is probably far less true for the psychoses.

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DISCUSSION

LEWIS: I think psychiatry has been a happy hunting ground for speculative endocrinology until very recently. Isolated observations don't really help very much, because they are equivocal, and one

I think the strongest evidence of our ignorance of many of these matters lies in the so-called involutional psychoses, which by their

irritability, negativism and contrariness. One woman with adolescent children became impossible to live with because of this, and the relatives stated that adrenal cortical extract restored her former sweet personality. Patients with Addison's disease may have neuroses, but I have not personally met with psychotic behaviour except in crises or terminal phases. Involuntary cries or grimaces are also met with in crises, and the patient tends to hide under the bedclothes or to become very restless and irritable.

Eunuchoid patients are usually of placid, docile temperament, although they may "flare up" occasionally. One was a good stonewaller at cricket; another was described as always quiet natured, and would never argue or fight. Two of them, who stated that they had no interest in the opposite sex, nevertheless married because their parents thought it would be a good thing and arranged the marriage. One of these kissed his wife as a routine, but without any affection or warmth. Coupled with rare and ineffective attempts at coitus, this was sufficient for his wife to leave him. The second patient was very similar in attitude, but also went to sleep in an armchair every afternoon till bedtime. Although some are depressed, they are seldom psychotic. Some educated eunuchoids may show fine intellectual powers and attain important positions. They may be interfering or intriguing.

Of the effect of testosterone in eunuchoids, I wrote in 1938: "Psychological and personality changes after testosterone are remarkable. Shyness and diffidence are lost. Patients become extroverted, creative, energetic and sometimes aggressive. They develop initiative and are capable of assuming responsibility. Depression and apathy disappear and may be replaced by euphoria.

One male patient aged 20, castrated by an accident at 16, slept a great deal during the day and was discharged from every job because of laziness, failing to get on with other people and because of touchiness. During the consultation, as at other times, he cried easily without obvious cause. He was said to have changed and to be like a temperamental female. He showed considerable charm of manner at times. Another surgical castrate was listless and apathetic, had no

ALLEN: They were occasionally rather aggressive weren't they?

ZUCKERMAN: Yes, indeed. They provided certain military leaders. But again, there is no indication that a castrated soldier is necessarily any the worse a soldier for being castrated.

frequently among them than among the ordinary population.

psychosis.

PINCUS Except for the fact that there are enormous fluctuations in adrenal activity, we don't know much.

there is no clear connection

LEWIS What I think Dr. Simpson meant by "minor" endocrine disorders are endocrine changes which can't be demonstrated, and I can't see any use in thinking about them until one has found methods of demonstrating them, or at any rate of finding a constant association between what one regards as the signs of them and the mental side of the question.

ZUCKERMAN. Is it Dr Simpson's thesis that all major endocrinopathies are associated with some change in behaviour or mental state of the individual?

SIMPSON No, it isn't. I said that they are not rare.

In answer to Dr. Lewis, I don't mean by "minor" endocrine disorders those which we can't demonstrate. On the contrary, I exhibit them whenever I get the cases—and there are many of them—and I think most people are coming round to the view that instead of saying "This is a major endocrine disorder, and it must have ten points, and if it hasn't got the ten points, it isn't one," they admit "We accept nine, we accept seven; we accept five; we accept four, and in some cases we

The point is

me pathology.

cognize the genetic

politically minded.

had not then presented any discernable change in her psychiatric picture, but a spontaneous rise of 17-ketosteroid and cortin excretion had started. This excretion rate continued to rise and the patient improved; finally showing a complete recovery. The thyroid activity was once again assessed, found to have fallen, and was within the normal range. Similar changes were seen in cases of anorexia nervosa, which also recovered after hospitalisation only (Reiss, 1942).

Before judging which changes are of primary importance in these cases, further investigation results should be considered.

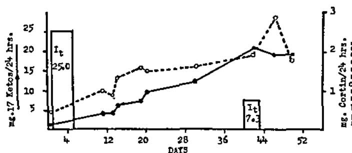


FIG 1. 17-Ketosteroid and cortin excretion during spontaneous mental improvement in a woman with puerperal depression

Fig. 2 shows a representative example of a depressive patient who recovered after testosterone and œstrone treatment. This patient had a negative adrenal cortex response to ACTH, a very low 17-ketosteroid excretion, and an elevated thyroid activity. She showed no spontaneous changes before commencement of treatment. Shortly after the beginning of the testosterone treatment, the ketosteroid and cortin excretion level rose, and after a month the thyroid activity was considerably decreased and the mental picture improved.

When there was no change either in the ketosteroid output, cortin excretion, or in the thyroid activity, after testosterone treatment, the patient showed no mental improvement. In this connection the investigation results shown in Fig. 3 are particularly interesting. It is the case of a depressed patient

PSYCHOLOGICAL CHANGES CONNECTED WITH SPONTANEOUS AND EXPERIMENTALLY PRODUCED ALTERATIONS IN STEROID HORMONE METABOLISM

MAX REISS

There are very few established facts known concerning the influence of hormones on the human brain, and any generalisation based on these would be dangerous. It is very difficult to standardise the conditions for any investigation in this field in order to draw valid conclusions. Animal experiments are much simpler, permitting the evaluation of a satisfactory number of controls for comparison with the results on treatment. In investigations on humans, however, the individual constitutional factors play such a decisive part in the outcome of the response that it is particularly difficult to obtain non-biassed or sufficiently representative samples, without which, even the most elaborate statistical evaluation becomes meaningless.

It might, therefore, prove more productive to study only individual reaction types in detail and to see afterwards what conclusions of general interest might be drawn.

In Fig. 1 some investigation results are recorded which were obtained during spontaneous improvement from mental disturbance; the patient was transferred to the mental hospital showing severe signs of puerperal depression. The thyroid activity of the patient was well above the normal range and the 17-ketosteroid and cortin excretion considerably below the normal range. While waiting for a decision on the most suitable method of treatment, determinations of 17-ketosteroid and cortin were carried out. Some days after the beginning of these investigations, it was decided not to proceed with any special treatment, although the patient

who improved in the first year very quickly after the commencement of testosterone treatment, while his ketosteroid and cortin excretion rose. Unfortunately, this treatment was discontinued after some time, and 18 months later the patient was re-admitted to hospital with signs of severe depression. Considering the beneficial results achieved in the first year, he was again treated with testosterone, but no change took place this time in the ketosteroid or cortin excretion, and the patient showed no mental improvement. In these and other similar cases, it would be tempting to

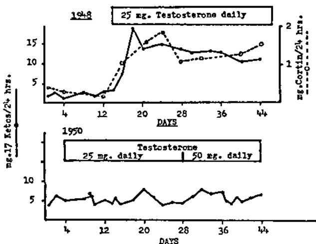


FIG. 3. Effects of two testosterone treatments on the 17-ketosteroid and cortin excretion of a male depressive patient.

consider what happens to the injected testosterone. The answer is obviously that it is being destroyed, similarly, perhaps, to the steroid hormones produced endogenously in the body, this being the cause of the low steroid hormone excretion rate. One might even go so far as to assume that

secondary changes as an underfunction of the gland. Such

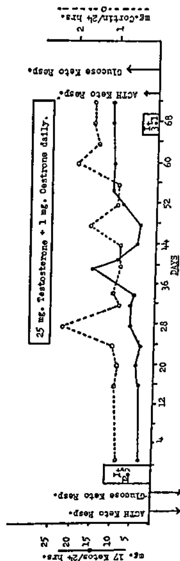


FIG. 2. Effect of testosterone and œstrone treatment on the 17-ketosteroid and cortin excretion of a female depressive patient.

considerations are also important in judging some of the actions of ACTH.

Fig. 4 shows changes in the ketosteroid and cortin excretion rate of a 30-year-old woman who was in a state of depersonalisation and confusion. The patient gave a positive ACTH-ketosteroid response, but a negative glucose-ketosteroid response and was, therefore, treated with 25 mg. ACTH b.d. The ketosteroid and cortin excretion rates were moderately increased after treatment, and what would appear most decisive, remained so during treatment. In this case there was a complete mental recovery.

Older women suffering mainly from depressive diseases are considerably more sensitive to ACTH than younger women. A daily injection of at least 20 mg. ACTH is needed in order to influence the ketosteroid and cortin excretion of the latter, while in the case of the older women, doses of 2–3 mg. per day are sufficient to bring about changes in their steroid hormone excretion. If they do not react in this way to the small dose, they very often do not react to the large dose either. If they do react, they show mental improvement, similar to that described by Hemphill and Reiss (1942), after small doses of ACTH.

In general, we have found male patients to be less sensitive to ACTH than female patients, at least with respect to the steroid hormone excretion indices of adrenal response.

When the steroid hormone excretion of schizophrenic
of excretion pattern
ely constant rate of
very young, simple,
acute psychological
changes, or by chronic patients who may be described as "burnt out" schizophrenics; secondly, an excretion rate showing day to day variations of several hundred per cent associated with schizophrenic patients who are very variable in their mentation, hallucinations and reactivity (Reiss, Hemphill, Gordon, Cook, 1949).

We have found that chronic mental patients often show a negative or limited response to ACTH with respect to the excretion of ketosteroids and cortin. This general observation is in agreement with the results described by Pincus and

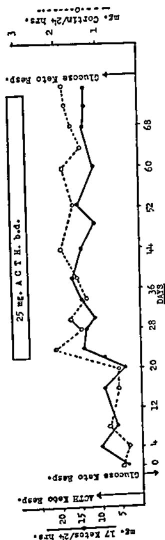


FIG. 4. Effect of ACTH on the 17-ketosteroid and cortin excretion of a 30-year-old woman in a state of depersonalisation and confusion.

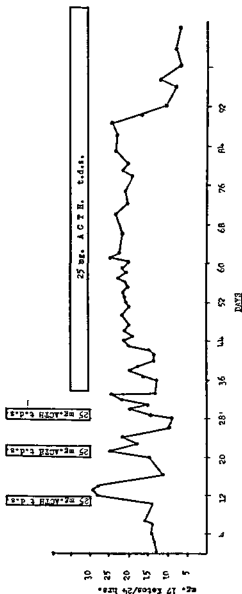


FIG. 5. Effect of ACTH on the 17-ketosteroid and cortin excretion of a 25-year-old male in catatonic stupor.

Hoagland (1949), on schizophrenics only. Some may, after application of large doses, show sudden and considerable rises in the excretion rate, but the level then drops more or less suddenly in spite of continuation of the treatment, and no change in the mental pattern is seen. One gets the impression that after these sudden rises, the adrenal cortex becomes exhausted, and cannot be further stimulated by ACTH. In other more acute patients, considerable changes in the excretion rate and mentation can be seen after ACTH. A very interesting example is seen in Fig. 5. This is a 25-year-old patient in catatonic stupor; his cortin and ketosteroid excretion varied. He was given 25 mg. ACTH for two days and immediately after the first injection the cortin and ketosteroid excretion rates rose far above his previous average level. Between 12 and 24 hours after the first injection, the previously mute and catatonic patient got up, started to talk, shaved himself and showed quite a [illegible] As soon [illegible] excretion [illegible] catatonic stupor. This treatment was repeated twice; each time the same sequence of events took place, the steroid hormone excretion rose and the patient improved mentally. ACTH was then given daily, when the ketosteroid and cortin excretion level remained high, and few changes were seen in the patient for 8 weeks, until suddenly, in spite of the continuation of the ACTH treatment, the ketosteroid and cortin excretion started to drop. A few days later, the patient became aggressive and increasingly unmanageable, further urine collection was impossible, and treatment and further investigation stopped. Similar unsatisfactory experiences were encountered, during the last 3 years, with a number of other acute male schizophrenics who were treated with ACTH.

Testosterone treatment occasionally proved more promising, particularly when the ketosteroid and cortin excretions were initially low, the thyroid activity slightly increased, and the ketosteroid response of the adrenal cortex to ACTH negative.

It was particularly interesting to follow up the steroid hormone excretion and mental changes of a manic-depressive patient on whom continuous investigations have now reached

together with testosterone; the growth of beard continued and mental improvement started simultaneously. This lack of sensitivity towards steroid hormones is only one example of peripheral under-sensitivity towards hormones in schizophrenic patients. It falls into the same category as the decreased sensitivity to insulin or the lack of peripheral sensitivity to thyroid hormone. Such states have been assessed on patients in our department, when normal or enhanced values for thyroid activity, as found by radioactive tracer techniques (Reiss, Haigh, Hemphill, 1952), are associated with decreased Basal Metabolic Rates. Also, in such cases, nicotinic acid or Priscol occasionally improve the paradoxical relationship between gland activity and sensitivity of the target organ. Such states of primary peripheral hormone insensitivity may bring about a later hyperfunction of the gland concerned.

The nature of the response of the adrenal cortex to endogenously produced or injected ACTH is apparently associated with the problem of peripheral insensitivity mentioned above. The responsivity of the adrenals is a decisive factor for endogenous changes in the thyroid function. The changes seen in thyroid activity after electro shock differ in accordance with the degree of adrenal cortex-ketosteroid response to ACTH injected or produced endogenously.

It would be a mistake to regard any one particular change in the excretion rate of steroid hormones as an essential feature of mental change or of mental recovery of patients. In examples mentioned above, mental recovery was usually accompanied by an increase in the excretion rate. If, on the other hand, mentally disturbed patients were investigated, where an adrenal cortex adenoma had to be removed, one finds, in agreement with Broster and his co-workers (1938), that the mental improvement is accompanied by a decrease in the ketosteroid excretion. The existence or re-establishment of the normal hormone level and normal ability of the gland to supply the normal amount of hormone, whenever the necessity arises, is essential. This condition of normality does not depend upon the function of any one particular gland, but rather on the conditions under which the gland

their fourth year. In the first two years, the investigation results of the beta-ketosteroid and cortin excretion were particularly interesting; when depressed, the cortin excretion rate of the patient went down, while the beta-ketosteroid excretion showed a tendency to rise. During the change from depressive to maniacal phase, including the intervening period of normality, the cortin excretion rose while the beta-ketosteroid excretion decreased (Reiss *et al.*, 1949). An injection of 50 mg. ACTH given in order to assess adrenal cortex responsivity produced in this patient a very surprising phenomenon. He was, at the time, in a depressed phase and not due for any mental change, but 12 hours after the injection, the patient became maniacal, only to fall back within 24 hours into a deeper depression than before. Twenty-four hours afterwards he was in his normal state and remained in it for the next six months.

Excretion rates and their changes, as discussed so far, are only very approximate indicators of changes taking place in the steroid hormone metabolism of the body, as we do not yet know all the factors involved. The individual steroid components excreted by mental patients occasionally vary considerably from the normal from a qualitative point of view. For instance, chromatographic analysis of ketonic material excreted by chronic patients reveals a much poorer pattern of steroid components than the normal. The complete analysis of the various components, if successfully carried out on a sufficiently large number of cases, should supply us with most important pathogenetic information in some groups of mental disturbances.

In other cases, the important factor is the sensitivity of the different tissues towards the steroid hormones. Some young schizophrenics excrete ketosteroids on a normal or even increased level, but do not show completely developed secondary sex characters, in particular lack of growth of beard. Recently, we had the opportunity of treating such a patient with high doses of testosterone. After 4 weeks' treatment, no growth of beard was seen. The treatment was continued with the addition, however, of the vasodilator nicotinic acid. After one week, beard growth was clearly recognisable. Priscol was later substituted as vasodilator,

Browne: I think Dr. Reiss is entirely right in his advocacy of careful study of single cases, rather than collecting fewer observations on a large number of cases.

I was interested in the rise in cortin under testosterone. Did you [illegible]

[illegible] in its output. In your instance the chemically determined corticoid went up, and in the other instance the biologically determined ones went down.

Reiss: We have found a group of people whose cortin excretion rate does not rise after testosterone, and another where the cortin excretion only starts to rise after testosterone treatment has been stopped.

Browne: I would like to ask Dr. Reiss whether if he goes on giving ACTH, as we know Thorn has in using a priming dose, do some of these patients show a response after a while or do they continue to be resistant?

Reiss: Initially disturbed adrenal cortex responsivity very often improved, parallel with the mental improvement. We have seen this after electro-shock treatment and, occasionally, after testosterone treatment.

Pincus: I am a very simple-minded fellow, and it seems to me that when you find a low 17-ketosteroid output and a low corticoid output that there is something wrong with the secretion of the adrenal. Therefore, one would suspect that if you gave ACTH you would increase the excretion of these compounds, and that is what Dr. Reiss has observed. For that reason, I am a little puzzled as to why one has to do more than accept the data on their face value, and I am perfectly willing to accept them as such.

[illegible]

sort of work that Dr Reiss has done, and I am very glad to see that he is doing it—it should be done. But the question whether the [illegible]

thyroid requires techniques which, I think, on the whole need further development. The thyroid I'm not quite so certain about. The

operates, for example, on the state of equilibrium between the different ductless glands. In this respect the relationship between the chief antagonists, adrenal cortex and thyroid, is of the greatest importance, and is shown to be so, by the very different action of a given dose of ACTH or steroid hormone on a hyper-thyrotic patient compared with that on a hypo-thyrotic one.

It would appear futile to draw conclusions on alarm or stress reactions, or the cause of mental changes, from the viewpoint of the function or the responsivity of a single gland, such as for instance, the adrenal cortex. Only analysis of the total endocrine equilibrium can enable us to explain why some patients show maniacal, euphoric changes or mental improvement after ACTH, while others show mental deterioration and psychotic changes, and yet others remain uninfluenced. In the present state of our knowledge, it might prove productive to study more carefully the pathophysiology of the single individual case.

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DISCUSSION

BROWNE: What was the method for cortin determination?

REISS. The usual chemical method for determination of cortical steroids, as described by Heard and Sobel. We deviated from this method only by using the Hagedorn-Jensen method for determination of the reducing power. Another method has been worked out lately which permits measuring colorimetrically the reduction of ferricyanide.

OBSERVATIONS ON THE MECHANISM OF THE DISORDERED PSYCHOLOGICAL OUTLOOK IN ADDISON'S DISEASE AND HYPOPITUITARISM

R. A. CLEGHORN

THE word mechanism in the title of this paper implies a finiteness of knowledge we do not have. It is, however, not altogether out of place, as it indicates the objective which, with more work and at a later day, may be achieved.

In his original description of the disease, Addison (1855), noted the occurrence of delirium. Later writers of that century added observations on localized neurological symptoms (Greenhow, 1875; Klippel, 1899). Phillips (1912) described a case with depression and delusions. Rowntree and Snell (1931), in their review of 108 cases, devote two-and-a-half pages of their 300-page monograph to nervous and mental symptoms. It is, unfortunately, impossible to get any idea of the incidence of the apathy, irritability, hallucinations and delusions and other central nervous system manifestations of which they speak. They considered the mental symptoms to be in all probability a manifestation of exhaustion, in which cerebral anæmia played a large part.

Early reports on the use of adrenal cortical extract were enthusiastic about its effect in reversing the adverse mental changes (Hartman, Beck and Thorn, 1933; Hartman, 1935), but later experience was not so favourable.

The nature of the metabolic disturbances in Addison's disease was fairly well worked out in the thirties, including the renal diuresis of sodium chloride and water, and retention of potassium and nitrogenous constituents (Loeb, 1942). The impaired carbohydrate metabolism which accompanies these changes was also described (Long *et al.*, 1940; Thorn, *et al.*, 1940). The use of sodium chloride and of

OBSERVATIONS ON THE MECHANISM OF THE DISORDERED PSYCHOLOGICAL OUTLOOK IN ADDISON'S DISEASE AND HYPOPITUITARISM

R. A. CLEGHORN

THE word mechanism in the title of this paper implies a finiteness of knowledge we do not have. It is, however, not altogether out of place, as it indicates the objective which, with more work and at a later day, may be achieved.

In his original description of the disease, Addison (1855), noted the occurrence of delirium. Later writers of that century added observations on localized neurological symptoms (Greenhow, 1875; Klippel, 1899). Phillips (1912) described a case with depression and delusions. Rowntree and Snell (1931), in their review of 108 cases, devote two-and-a-half pages of their 300-page monograph to nervous and mental symptoms. It is, unfortunately, impossible to get any idea of the incidence of the apathy, irritability, hallucinations and delusions and other central nervous system manifestations of which they speak. They considered the mental symptoms to be in all probability a manifestation of exhaustion, in which cerebral anæmia played a large part.

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deoxycorticosterone acetate in therapy was defined. The adrenal cortex as a source of androgenic compounds of importance in protein metabolism was also recognized (Albright, 1942).

The intensive work on the metabolic functions of the adrenal cortex had to precede interest in the psychological aspects of the disease. The simultaneous development of psychiatric interest, in what had hitherto been considered exclusively medical cases, accounts for the gradual focusing of attention on mental disturbances which often accompany this disease. Individual cases showing a psychosis were described by Porot (1937), Larue (1938), Rushton *et al.* (1940), Chatagnon *et al.* (1942). Wider recognition of the high frequency of psychologically abnormal symptoms appeared with the publication of papers by Engel and Margolin (1941, 1942). They found that 16 of 25 Addisonian patients admitted during a 10-year period to the Mount Sinai Hospital showed significant deviations from normal. Three of their cases were frankly psychotic. My own experience supports that of Engel and Margolin. A summary of some personality characteristics of 25 cases seen in a 10-year period is contained in Table I. This information must be considered impressionistic as it is derived from recollection, not an accurate method, but it does attempt to give an overall picture. It may be added by way of extenuation that neither Soffer (1946), nor Thorn *et al.* (1949*a*) have emphasized or itemized from their wide experience the psychological abnormalities met in their cases, though they make reference to the frequent occurrence of such abnormalities.

The estimate of the psychological picture, in the cases in Table I, is not meant to be that of their status in severe or even mild adrenal insufficiency. It represents the patients' outlook when on treatment with sodium chloride and deoxycorticosterone acetate; electrolytes and tissue fluids thereby being controlled. It should be added that of these 25 cases, one man and one woman could be considered fairly vigorous and well adjusted. One other young man had a cheerful attitude in marked contrast to most cases. He had a severe pre-existing diabetes. This casual observation acquired added significance when it was learned that Thorn

and Clinton (1943), had described an alteration in the disposition of a case of Addison's disease with the development of diabetes. Previously shy and taciturn, he exhibited more spontaneous enthusiasm after the onset of the diabetes.

Table I

PSYCHOLOGICAL ASPECTS OF ADDISON'S DISEASE
25 cases: 10F, 15M (Cleghorn)

	No	Per cent
Apathy (psychic)	21	84
Negativism	20	84
Seclusiveness	12	48
Depression	12	48
Irritability	12	48
Suspiciousness	4	16
Agitation	2	8
Paranoid with delusions . .	1	4

In 1947, Gorman and Wortis added two more cases of paranoid psychosis in Addison's disease to the record. Both showed laboratory findings characteristic of the disease. The first, a 28-year-old female, had been known to suffer crises of adrenal insufficiency for 7 years. She became irrational only in one of her late crises, and became normal overnight with DCA. With the accidental interruption of this therapy, she became delusional, paranoid, hallucinated, disoriented and defective as to memory, retention and calculation. She died in a week despite therapy. The second was a 61-year-old woman with a 4-year history of the disease before admission. Just 5 days prior to hospitalization she stopped DCA injections and began to act queerly. She
was evasive,
no memory

though it was known death occurred ten months later. These authors stress the importance of electrolytes and carbohydrates for brain metabolism, and imply that physiological disturbances in these may cause the psychotic process. [illegible] that " the victims of a chronic debilitating [illegible] from fractures to cardiac decompensa[illegible] paranoid psychosis.

A fully documented case from the service of Dr. J. S. L. Browne had had Addison's disease for at least 4½ years when mental symptoms appeared at the age of 33 in 1949. She had previously been [illegible] apprehensive and worrying kind of [illegible] therapy, had carried on at a reduced [illegible] for her husband and child. In late summer, 1949, about 10 months after a pellet implantation, she began to have renewed Addisonian symptoms, but also became hallucinated and paranoid. Fresh pellets were implanted, but unlike Gorman and Wortis' (1947) first case, her hallucinations and paranoid state persisted. Four months later, a Rorschach test disclosed a "rigid, impoverished, meticulous and perfectionistic individual, who presents evidence of considerable anxiety, sexual conflict, and possible paranoid trends." Clinically, she was anxious, depressed, deluded and paranoid at this time, despite relatively normal electrolyte values. Her later progress was favourable with other therapy.

Mental Changes in Hypopituitarism

The major metabolic defects in hypopituitarism result from the involvement of the thyroid and adrenal cortex, and have been ably summarized with respect to that most severe form, Simmonds' disease, by Escamilla and Lisser (1942), Sheehan and Summers (1949), and Farquharson (1950). Therefore, the anticipated mental changes are those associated with hypofunction of either target gland, or both.

There is almost always a disintegration of the personality to some extent. This is manifested by inability to work or concentrate, slowness, irritability, depression, negativism and even paranoid ideas. These cases resemble myxœdema

more often than Addison's disease. The common hypoglycæmic episodes explain, to some extent, periods of mental confusion, convulsions and attacks of coma. Farquharson says that intellectual impairment is worse in severe cases than in severe cases of myxœdema, with the additional element of loss of judgment, and more frequently, personality changes. Psychoses may occur in hypopituitarism, as they may in Addison's disease and as reported for myxœdema (Asher, 1949.

The aspect about which there is so little data available is the mental state of the patient when electrolyte and blood sugar values are controlled to lie within normal limits. The recently reported case of Schrock *et al.* (1951), had psychotic features. Thyroid extract had been of no help; indeed, it had precipitated an Addisonian crisis. Hallucinations were present when the adrenal cortical factor was controlled by extract injections and the sodium and potassium levels were not far off normal.

More intense psychological studies have been undertaken in several cases of hypopituitarism of Dr. J. S. L. Browne, and he will speak of these himself.

Conditions Affecting the Electroencephalogram in Addison's Disease

As mentioned above, the frequent occurrence of mental symptoms in Addison's disease was clearly recognized by Engel and Margolin (1942). They were also the first to show that there was an abnormality in the E.E.G. in a high proportion of these patients (1941), five out of eight, a finding confirmed by Hoffman *et al.* (1942) in 18 out of 25 cases. The characteristic picture consisted of three to six waves per sec., of a voltage higher than alpha, which often appeared in bursts. Hyperventilation produced slow high voltage waves more easily than in normals, also low O_2 tension. A rough correlation to blood sugar levels existed. Glucose protected somewhat against the slow waves of hyperventilation. In normals, insulin hypoglycæmia may produce such waves [illegible] cent (Himwich *et al.* ([illegible] r level due to diabete[illegible] (Thorn and

Clinton, 1943), still showed an abnormal E.E.G. pattern, though there was an improvement in mood, as already mentioned. DCA treatment did not abolish the abnormalities in the majority of instances, though the sensitivity to hyperventilation was decreased in some. Engel and Margolin (1942), thought that Vitamin B therapy improved the E.E.G., while Hoffman *et al.* (1942), and Engel and Romano (1944), did not. The latter gave 100 ml. ACE over five days with fairly effective restoration of the E.E.G. pattern. DCA did not do this. More recently, a report has been made by Thorn and others (1949*b*), that cortisone restores abnormal E.E.G. patterns. F. Engel (1949) reports Scheinberg as having found no change in cerebral oxygen consumption or glucose utilization, as shown by the Kety method, in two cases of Addison's disease and one of hypopituitarism. One patient was actually showing hypoglycæmic symptoms at the [illegible] (Thorn *et al.*, 1940). Glucose and cortical hormone intravenously caused only a small decrease in oxygen consumption.

Experimental confirmation for the alteration of the E.E.G. pattern has been provided by the Worcester Group. Bergen (1951), in a recent publication, has found that the frequency spectrum of E.E.G. waves was shifted by adrenalectomy from 50 per sec. to a slower rate of 40 or less. DCA did not increase the rate, whereas lipoadrenal extract, which contains relatively large amounts of carbohydrate active agents, restored it towards normal. A similar effect was obtained with Δ^5 pregnenolone. Since DCA is ineffectual in correcting E.E.G. changes, it seems unlikely that correction of electrolyte changes are of major importance.

Brain Excitability in Relation to Steroid Hormones

Another technique for investigating changes in brain function in relation to the adrenals has been elaborated by Woodbury *et al.* (1950, 1951). They have measured the minimal amount of current necessary to induce clonic convulsions in rats, which they call the electroshock threshold.

It is a measurement of excitability. In intact rats, DCA rendered the rat less sensitive, i.e., increased the electroshock seizure threshold (EST). This can be counteracted by adrenal cortical extract (Woodbury *et al.*, 1950), and cortisone (Woodbury *et al.*, 1951). In intact and adrenalectomized rats implanted with DCA and given water or 0.9 per cent NaCl to drink, the thresholds varied from lowest to highest as follows: intact controls on water; next, intact DCA rats on water; next, adrenalectomized DCA rats on water; next, same on saline. ACE and cortisone lowered the EST of DCA saline treated rats in 6 days with a return to pre-treatment levels in 11 days. In the DCA rats on water, the effect of cortisone occurred in 3 days, and the return took place in 2 days. The authors interpret these findings as indicating (1) that intracellular Na is an important factor in determining brain excitability, and (2) even more important, that DCA competes with cortisone-like steroids for the sites of action of the hormone. In other words, it is possible that being so similar in structure, they "compete for the strategic loci in the cell." In the intact animal, DCA has another action, that of inhibiting pituitary adrenocorticotrophic activity. The explanation of the more rapid return of EST in the DCA water rats after cortisone withdrawal cannot be explained on the basis of Na retention.

Summary

Abnormalities of personality develop in people with Addison's disease. At present, it cannot be said with certainty that these are specific to the disease or just an accompaniment of a debilitating state. There is an equal chance that the psychosis which occasionally occurs is fortuitous. It should be noted, however, that there are no indisputably proven cases with psychosis antedating the onset of adrenal deficiency, despite statements in the literature to the contrary. Usually, the mental symptoms are worse when electrolyte, water and carbohydrate disturbances are present. Adequate control of water and electrolytes by DCA and ingested salt and high carbohydrate intake does not eliminate many mental symptoms, however. The replacement is inadequate. Adaptation to stress is poor.

Hypoglycæmic symptoms occur at blood sugar levels tolerated well by normals. Abnormal E.E.G. patterns persist. These lines of evidence indicate that the mental disturbances occurring in this disease may depend on a deviation of metabolic functions other than electrolytes and water. Evidence from the use of cortisone in this disease supports such a conclusion. It is imperative that more attention be paid to the person with the disease and that psychiatric assessment of the case throughout be the objective, if more is to be learned about hormones and behaviour.

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DISCUSSION

Simpson: A large group of those symptoms, apart from the last few, suspicion, agitation and paranoia, I think have been very well known and described—I described them thirteen years ago, and I believe other people did before.

behaviour, or even psychosis, because in comparable fashion either low

You get the characteristic involuntary cries and grimaces and a tenden-
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somewhere between 80 and 100 cases of Addison's disease and I don't happen to have come across an actual psychosis except in the terminal phases where the treatment has gone wrong or a severe infection has turned the balance adversely.

BEACH: During the war an experiment was conducted at the University of Minnesota to determine the effects of inanition on healthy young men. For six months the daily caloric intake was held at about 3000, and it was then reduced to around 1200-1800 for six months. As I recall the results, on this reduced caloric intake some of the subjects exhibited most if not all of the symptoms that Dr. Cleghorn listed in his first table, with the exception of paranoia. I mention this because it points up something that has impressed me as we listened to several papers this afternoon. It is the compelling need for a more accurate analysis and understanding of the psychological side of this problem. We are gathered together to talk about relationship between endocrine functions and behavioural functions, but with few exceptions we seem to be much more sure of ourselves on the endocrinological side than we are on the psychological side Dr. Cleghorn has mentioned the need for more accurate and sensitive endocrinological tests, and I would hope for better tests on the psychological side. For example, I am sure that Dr. Cleghorn will agree that reminiscence is a very poor substitute for such a test. Our need is for tests that can be carried out in a standardized fashion during the time that the endocrinological analyses are being made.

CLEGHORN. I would agree.

BROWNE: I would entirely agree. We have found sometimes that even using a battery of psychological tests and using also the psychiatrist as an observer that the reaction of the patient to the psychiatrist himself

ZUCKERMAN: I take it that the amount of correlation between the

BROWNE: I agree with you. It seems to me that the problem with

ALLEN: Were there any visual hallucinations in any of Dr. Cleghorn's cases? Dr. Simpson suggested a sort of toxic psychosis; and in toxic psychosis visual hallucinations are quite common, whereas in other psychoses they are very rare.

ALLEN: I would distrust visual hallucinations described as visions by anybody other than psychiatrists, because it just doesn't mean anything. People say "He's got visions," and then when you ask them, they say "He hears voices." Even physicians say such things.

BROWNE: Tomorrow, I will talk about a case of Addison's disease treated with cortisone; it came out in the most dramatic manner I have ever experienced. This is a case where the various psychiatrists disagreed.

ZUCKERMAN: To what extent do psychiatrists disagree about their diagnoses? To the same extent as physicians?

LEWIS: I don't think diagnosis comes into it. I think observation is required; diagnosis is unimportant here.

BROWNE: What I said was not correct. The various physicians, including a psychiatrist, did not agree.

ZUCKERMAN: About what? About the observations or about the diagnosis?

BROWNE: About the actual existence of these hallucinations, when they appeared, and so forth. Dr. Cleghorn was the psychiatrist and he will discuss it.

high.

LEWIS: We used the scale worked out at Worcester, and have found that the correlation between different doctors and also sisters of wards, was very high indeed.

KALMUS: In relation to what Dr Beach said, I should like to point to

seem to be great differences between people, and also, in women,

BROSTER Addison in his original articles decribed "cerebral attacks," and in our cases we have had two with very definite neurological symptoms. In one the attack passed over; in another, a boy of 16

ELLIOTT: It was a peculiar picture, a boy whose blood sugar and chemistry were completely normal, with slow waves in the E.E.G I

a weird condition which I could not correlate with any biochemical abnormality at the time. I don't know if anybody else has seen it.

CLEGHORN. That has been described by Engel and Margolin, and earlier by Klippel in 1899. But I think you are quite right, that one cannot detect in a case like that biochemical abnormalities. If you had had cortisone to treat that case with, the abnormal waves might have cleared up.

ZUCKERMAN· Do you draw any conclusions from the fact that the blood chemistry was normal? That there was no derangement of metabolic function?

ELLIOTT: I only conclude that the factors which were normal couldn't have been relevant to his condition.

ZUCKERMAN: Did you make any observations on ketosteroid excretions?

ELLIOTT: No.

SIMPSON Was the blood chemistry normal at the time of the initial attack?

ELLIOTT: Yes.

BROWNE: In a case of pituitary *insufficiency, drowsiness and coma* occurred at higher levels of blood sugar from year to year: 6 years ago, the blood sugar was 22 mg per cent; three years ago, 50; and last year, 80.

CLEGHORN: This is also true of Addison's disease.

ELLIOTT: Many severe diabetics experience hypoglycæmic symptoms if the blood sugar is induced to fall to quite moderate levels—levels at which a normal person would show no such symptoms.

GREENE: On the other hand, there is the confusing fact that occasionally in Addison's disease you get what is, I suppose, a hysterical perpetuation of the hypoglycæmic coma. I had a case, for instance, of a man who was extremely well controlled from the point of view of his electrolyte metabolism, but who used to return to hospital in a coma, apparently deep coma, at intervals of a few months. He was taken to the Casualty Dept., and was immediately treated with glucose drip *and large quantities of eucortone. Then I observed that these attacks* always occurred on Thursday afternoon, which is the afternoon I happened to be in the hospital. So I gave instructions that he was

ADRENAL FUNCTION IN SCHIZOPHRENIC MEN*

GREGORY PINCUS

In this paper I propose summarizing briefly our recent studies of the pituitary-adrenal mechanism in schizophrenic men and non-schizophrenic subjects. In addition, data on aspects of steroid excretion in schizophrenic and control subjects will be presented.

Pituitary-adrenal Mechanisms in Man

In Figure 1 we present diagrammatically the pituitary-adrenal mechanism in men and the tests that we have employed to analyse the various components of the system. As indicated in previous publications (Pincus *et al.*, 1949*a*, *b*; Pincus and Hoagland, 1950; Hoagland, 1950), identification of the locus of a defective response to stress may be attempted with these tests since: (*a*) the administration of corticosteroid (ACE) tests end-organ responsivity, (*b*) the administration of adrenocorticotrophic hormone (ACTH) tests adrenal secretory potency, and (*c*) the various stress tests involve activation of the anterior pituitary to secrete ACTH. Analysis of the pituitary-adrenal mechanism by these tests has, in most of our studies, involved acute (4-hour) experiments, since the objective has been the unravelling of the mechanism rather than a study of long-term effects of either stress or hormone administration.

The administration of a potent adrenocortical extract to schizophrenic men is followed by lymphocytopenia, sodium and potassium diuresis, uricaciduria, 17-ketosteroiduria and corticosteroiduria in the first hour following administration

* [illegible] Depart- [illegible]
Agency.

with a continuation into the next $2\frac{1}{2}$ hours (except for the Na excretion). The responses indicate characteristic end-organ responses and, except for the later-hour Na excretion, are not significantly different from the responses given by normal men. The steroid output data are presumably measures of metabolites of the administered ACE and the

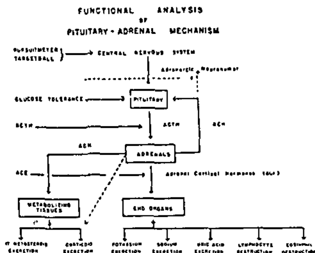


Fig. 1. The rôle of various experimental procedures in the analysis of the pituitary-adrenal mechanism

data for schizophrenic men differ significantly from the normal subject's data only in the later-hour 17-ketosteroid output. We have concluded from these data that defective stress-response observed in our schizophrenic subjects does not involve inability of the end organs to respond to active adrenal steroids.

Since the various stress tests employed and the administration of ACTH led on the average, in our schizophrenic population, to reduced responsivity by our measures of response, it was concluded that the major locus of the defective responsivity was the adrenal cortex itself, which presumably failed to secrete active hormone in normal amount.

A more detailed analysis of the individual responsivity of schizophrenic men disclosed that approximately 70 per cent

of the schizophrenic population in these studies was by these measures "hypoadrenal" (Table I). In undertaking this

Table I

THE TOTAL RESPONSE INDEX OF VARIOUS SUBJECTS IN THE STRESS TESTS

Test	Subjects	Mean TRI	Per cent of subjects with score of 20 or greater	Ratio of normal responders to schizophrenic responders
ACTH	Normal (25)	46.2	100 0	
,,	Schizophrenic (25)	12.0	28 0	3.6
Pursuit meter	Normal (46)	22.1	47 8	
,, ,,	Schizophrenic (36)	−2.7	15 8	3 0
Target ball	Normal (36)	11.5	32 5	
,, ,,	Schizophrenic (20)	−6 7	10.0	3 3
Glucose tolerance	Normal (47)	22 1	48 6	
,, ,,	Schizophrenic (38)	5.7	15.0	3.2

analysis, we employed a total response index (TRI) as a single quantitative measure of the indices of cortico-adrenal response. Quantitatively normal values of the TRI were obtained for approximately 30 per cent of the schizophrenics studied. It was noted, however, that the increased urinary output of neutral reducing lipids (NRL) following any of our test procedures was not significantly different in our control and schizophrenic groups. The ratio of NRL to our other indices in the schizophrenic subjects having quantitatively normal TRI values was significantly different from the corresponding ratios for the normal subjects (Table II).

The schizophrenic population analysed in our earlier studies was one having an average hospitalization period of two years. More recently we have studied responsivity to [illegible] and within [illegible] oportion of [illegible] s than 20), is somewhat less in this group (about 50 per cent compared with 70 per cent in our original group), but there is no significant correlation in our data between TRI and duration

of hospitalization. Furthermore, in the group of more acutely ill men, the ratios of NRL to other indices are again abnormal.

In considering the lack of responsivity to stress and ACTH, we examined the possibility that faulty nutrition might be the basis (Pincus *et al.*, 1949*c*). In an experiment involving

Table II

THE RATIO OF MEAN PERCENTAGE CHANGE IN NEUTRAL REDUCING LIPID OUTPUT TO THE MEAN PERCENTAGE CHANGE IN OTHER INDICES.

The data are those of the 14 schizophrenic men classified as positive responders to the 3 stress tests and a similar group of normal subjects (see text).

Response index	Ratio	
	Schizophrenic men	Normal men
17-ketosteroid output	1 12	1.35
Uric acid output	0.80	1 38
Potassium output	0.87	1 31
Sodium output	0.94	1.30
Lymphocyte number	0.61	0.82

the feeding of a high-protein, high-vitamin diet to a group of schizophrenic men, it was found that no significant increase in responsivity to ACTH could be induced in the "non-responders", nor was there any notable change in the data for the "responders" on this regimen.

Our interpretation of the foregoing findings was that steroidogenesis in response to ACTH was abnormal in schizophrenic men. Increasing the ACTH dosage led to TRI values closer to normal (Pincus *et al.*, 1949*a*), and non-convulsive electroshock led to similar results (Hoagland *et al.*, 1950), suggesting that secretion of active corticosteroid in physiologically effective quantity was possible, given adequate stimulus. The basal output of neutral reducing

lipid was consistently lower, on the average, in schizophrenic men despite the fact that percentagewise it may be significantly elevated by stress or ACTH administration. The suggestion was made, therefore, that the schizophrenic's adrenal cortex may be in the equivalent of the stage of *resistance of the adaptation syndrome (i.e., in a condition* resembling that of panhypopituitary patients), and that this may be characterized by the production of atypical corticosteroid. The fact that reduced basal NRL excretion was accompanied by normal basal outputs of 17-ketosteroid (Pincus *et al.*, 1949*a*), suggested the possibility of steroid dysgenesis.

Steroid Hormone Excretion Studies

The elucidation of steroid metabolism in schizophrenic subjects has been the objective of recent studies.

The development of improved methods for the extraction of urinary corticosteroids and of their chromatographic separation (Pincus and Romanoff, 1950) has made possible an initial study. In Table III we present data on the

Table III

THE MEAN CONCENTRATIONS OF URINARY CORTICOSTEROIDS (AS MG. 11-DEOXYCORTICOSTERONE EQUIVALENT PER GM. CREATININE) IN SCHIZOPHRENIC AND ARTHRITIC SUBJECTS.

Subjects	Number	Total neutral reducing lipids (NRL)	Total neutral formaldehydogenic steroids (FS)	NRL/FS
Schizophrenic.	9	4.51	0.58	7.7
Arthritic .	15	6.92	1.19	5.8

urinary excretion of corticosteroid measured as neutral reducing lipid (NRL), and as formaldehydogenic steroid (FS) in schizophrenic and non-schizophrenic (arthritic) subjects. As indicated by our former data (in which NRL extracted by a less adequate quantitative method was measured), the schizophrenic subjects excrete quantitatively less corticosteroid. This quantitative difference between the two sets of subjects is most marked in the FS excretion

since the control subjects excrete 105 per cent more FS than the schizophrenics, whereas they excrete 53 per cent more NRL. Our original suggestion that the schizophrenic [illegible] id [illegible] a [illegible] se same subjects to chromatographic separation using silica gel as the adsorbent (Pincus and Romanoff, 1950), in order to delineate more specifically the corticosteroid excretion pattern of our experimental subjects. The corticosteroid extracts were separated into ketonic and non-ketonic fractions before chromatography. Three major eluates are obtained: (I) Containing the least polar substance, typified by progesterone and deoxycorticosterone; (II) containing more polar substances such as corticosterone, cortisone and 11-hydroxycorticosterone, and (III) containing the most polar compounds, the nature of which is largely unknown, but concentrating presumably highly hydroxylated corticosteroids. The main data on these eluates in control and schizophrenic subjects, representing the proportion of the total measured found in these three major eluates, are as follows: the ketonic reducing lipid distributes remarkably similarly in the two groups, 52 to 53 per cent appearing in I, 30 to 31 per cent in II, and 19 to 20 per cent in III. Non-ketonic reducing lipid is not markedly dissimilar, being identical in II (25–26 per cent), not significantly higher for the controls (57 per cent) over the schizophrenics (52 per cent) in Group I, but rather low in the controls' data (13 per cent compared with 19 per cent) in III, suggesting a tendency for the schizophrenics to excrete relatively more of the highly polar compounds This tendency is again reflected in the data on ketonic FS, and is most marked in the data for non-ketonic FS. We hope in future work to be able to identify specifically the compounds involved in these excretion patterns, but these data suggest that highly hydroxylated α-ketols and glycols may be especially characteristic urinary corticosteroids in schizophrenic subjects.

We have also undertaken the investigation of the neutral urinary steroids of acid hydrolysed urines. The neutral

lipid has been separated into non-ketonic and ketonic fractions. Non-ketonic steroids have been measured by the application of the $SbCl_3$ reaction (Pincus, 1945). Again, the exact nature of the non-ketonic steroids which react as chromogens to $SbCl_3$ is not known, but androstanediol-3, 17 is quite chromogenic, pregnanediol much less so. In Table IV, data on the basal outputs ("pre-test") of these chromogens (expressed as mg. androsterone equivalent per g. creatinine) are presented for groups of normal,

Table IV

NON-KETONIC NEUTRAL LIPID, MG. ANDROSTERONE EQUIVALENT

Subjects	No	Pre-test
Normal men	13	6.7
Schizophrenic men	8	2 0
Arthritics—I	9	5.3
Arthritics—II	11	6 6
Schizophrenics (Cort)	8	3.1
Arthritics—I (Preg.)	9	6.9
Arthritics—II (Cort.)	12	3 3

schizophrenic and arthritic subjects Each of the subjects *received a test dose of 25 mg. of ACTH at the conclusion* of the 3-hour pre-test period, and the excretion of non-ketonic steroid was measured for the four-hour period following ACTH administration. It is clear from these data that these non-ketonic substances are, during the basal period, excreted in much lower amount by the schizophrenic subjects. Furthermore, ACTH administration does not cause any significant absolute or relative change in this output. It is interesting to note that arthritic subjects receiving cortisone (100 mg./day), exhibit a decreased output of non-ketonic steroids, whereas the schizophrenic subjects (receiving the same cortisone dosage) have no further depression of output.

The urinary ketosteroids in groups of schizophrenic and normal subjects have been fractionated into α and β components. Data of this study are presented in Table V. As indicated in our previous data, the total 17-ketosteroid

Table V

KETOSTEROID DISTRIBUTION IN NORMAL AND SCHIZOPHRENIC MEN

Subjects	No	Mean age	Total 17-ketosteroids mgm /gm creatinine	β-Ketosteroids mgm /gm creatinine	β Ketosteroids as per cent of total	$SbCl_3$ ketosteroids as per cent of total
Normal	13	29.0	8.38 ± 0.79	0.50 ± .062	6.39 ± 0.87	62.8 ± 4.46
"	14	54.2	4.70 ± 0.31	0.27 ± .031	5.88 ± 0.57	53.7 ± 2.64
Schizophrenic	21	34.6	7.11 ± 0.68	0.84 ± .135	11.43 ± 1.06	61.3 ± 2.49

output (measured by the Zimmerman, reaction), does not differ significantly when comparison is made between normal and schizophrenic men of similar ages. But a significantly elevated β-ketosteroid output is found in the data for the schizophrenic subjects. Aliquots of the same extracts measured for $SbCl_3$ chromogen show no significant difference

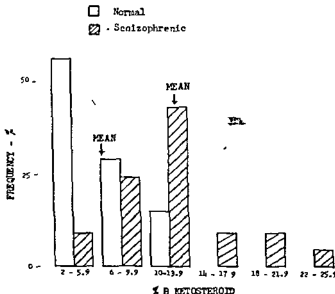
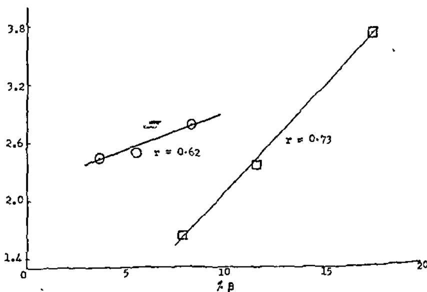


FIG. 2 Frequency distribution of the percentages of β-ketosteroids in the neutral ketonic fractions of urine taken from normal subjects (open rectangles) and schizophrenic patients (cross-hatched rectangles)

between schizophrenic and normal patients of the same age group (Table V, last column). Although the older men of this study exhibit reduced absolute outputs of both total and β-ketosteroid, the β-ketosteroids taken as a percentage of the total are essentially the same (5.88 per cent) as for the younger subjects (6.39 per cent). We have, therefore, compared the frequency distributions of percentage β-ketosteroid in the two normal groups with the distributions in the schizophrenic group. Figure 2 demonstrates there is an overlapping of these distributions, but a clear shift of higher values in the schizophrenic population.

In seeking to assess the significance of the last mentioned difference between the schizophrenic and normal subjects, we have found that corticosteroid output (as NRL) and per cent β-ketosteroids excreted are significantly correlated in both normal (r = 0.62), and schizophrenic men (r = 0.73).



As Figure 3 demonstrates, the slopes of the regression lines are, however, markedly different in the two groups of subjects, being nearly three times as steep in the schizophrenics' data. No other measures of steroid excretion correlate thus significantly. If we assume that the corticosteroid output measures with some fidelity the secretory products of the adrenal cortex, whereas the β-ketosteroids represent those corticosteroids which fail to become completely metabolised to α-ketosteroids, then the data of Figure 3 suggest a relative incapacity of the schizophrenics to effect conversion of certain precursors to α-ketosteroids.

Let us now summarize the excretion data findings. The schizophrenics excrete lesser amounts of corticosteroids and neutral non-ketonic steroids; among the corticosteroids the schizophrenics exhibit a higher proportion of more polar compounds; among the neutral ketonic steroids the β-ketosteroids of schizophrenics are disproportionately high. The suggestion of steroid dysgenesis is repeated. It is as if the schizophrenic subject secretes diminished amounts of corticosteroid which differ qualitatively as well as quantitatively from the secretory product of the normals' adrenals; the secretory product of the schizophrenics' adrenals is a poor precursor of non-ketonic steroid and of α-ketosteroid.

What is this schizophrenic product? Does it affect brain function? Or is it the result of brain dysfunction? These are questions we hope to answer.

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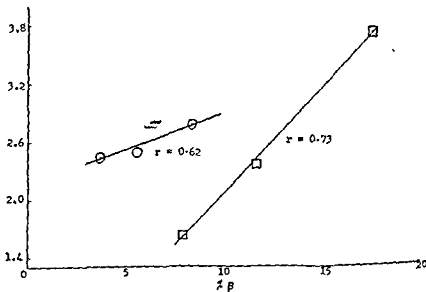
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... between the neutral reducing lipid

established some contact with them, so far as that can be done. And she found that while it was true that they did not respond to certain stress tests which you described, that if she was to establish enough contact with them to evoke in an interview certain previous emotional distresses which they had had, that the adrenal then did respond. In other words, it was a matter of penetration to the mechanism. That would more or less agree with the statement you have just made.

PINCUS I think that that is an excellent point. It is one that is not easy to answer, but perhaps I can give you some indication First of all, the patients we have used generally are pretty co-operative; on a number of other patients you can't do these tests. It's obvious that you have to do many things, give them injections, collect urine, manipulate apparatus, and so on, so that to that extent they are in contact. We are puzzled about the so-called responders. Clearly if there is a response to ACTH administration, it suggests that there is some adrenal secretory function there. And so we have considered two possibilities. One is that these effects are higher in this hierarchy and it might lie at the level of the pituitary or it might lie within the central

and these are our non-responders. In the others the ratio of active to relatively inactive is larger and these are our responders, and we can't decide between those two alternatives on any data that we now have Dr. Gildea, who repeated these experiments using both ACTH and stress, finds essentially that the response to stress is defective but the response to ACTH in his subjects by the method he used is fairly normal So there is no doubt, at least on the basis of all the data that I have seen, that there are differences between schizophrenics in terms of adrenal function. The basic question is, what is this difference, and all I have been able to give you today are some preliminary indications that there may be faulty steroidogenesis by the adrenal. There are other factors, in all probability, which we are hoping to be able to analyse.

LEWIS: In the course of previous investigations a lot of tests have been used and observations made which led to the conclusion that there is a general disturbance of homeostatic mechanisms in schizophrenics. Is there any relationship between the steroid findings and the findings of these other physiological and psychological observations?

DISCUSSION

... I'm a bit out of my depth here, and when you say six ... ow whether you are saying the ... While your figures, as you say, indicate a significant difference, the variance of your observations seems to be very much the same in both the schizophrenic and normal groups. It made me wonder how they were selected? I should have expected that so-called normal men are more of a kind than are schizoids. Am I wrong? Are schizophrenics all very much alike?

LEWIS: I'm sure that the investigators at Worcester, who have had exceptional experience of schizophrenia, would have selected in these six cases typical examples of schizophrenia, regarded as a mental illness with well recognized features and tending towards a chronic course.

ZUCKERMAN: To take a typical schizophrenic is something quite different from taking a schizophrenic at random out of a number of people diagnosed as such.

... measurements made on

BROWNE. Have you made any differentiation between symptomatically different schizophrenics, the catatonic ones and the non-catatonic?

... We have made very little attempt to analyse in that direction, ... which

BROWNE. ... as hearsay. ... been working ... She has been doing psychotherapy on them, phrenics

entity, is possible at present. Not only under-functional states of the adrenal cortex, but also over-functional states have been found by us to be connected with schizophrenia. As most essential, however, appears to us the *instability* of the adrenal cortex function of such patients, the disturbed equilibrium, a state which is responsible for the enormous day to day fluctuations in the output of ketosteroids and cortical steroids. In this connection, also, the rôle played by the big

physiology of mental disease.

After ACTH there is an enormous outpouring of phosphate in the urine of schizophrenics. This is in contrast to a slight retention in normals

hand, and normal and arthritic patients on the other, but is there any further differentiation that can be made? It seems that abnormal adrenal cortical function can be associated with the psychiatric diagnosis of schizophrenia, and also with a number of other conditions. Earlier this afternoon there appeared to be some doubt about the thesis that certain specific mental states were reflections of different endocrinopathies. Your paper, however, as well as Dr. Cleghorn's and Dr. Reiss's, all seem to indicate, in different ways, that there may be these specific associations. To a non-expert it nevertheless seems that, at least to some extent, you may be talking about the same thing, and I would like to see the endocrine picture dissected a bit more clearly. Are these states very different from the hormonal point of view?

PINCUS: You shouldn't ask such questions, because you are only embarking on speculation. I think all that Dr. Reiss, Dr. Cleghorn and I are doing are giving data on the patients. I don't think we are in a state yet where we can make any very pronounced differentiation I think that it is obvious on the basis of some of the recent literature particularly, that adrenal dysfunction characterizes many diseases. I am not at all sure, although we've observed it in the schizophrenics, that what we have seen is typical of schizophrenia. All I can say is

at sea. We are going to try to develop methods which will be more specific, to find out exactly what compounds characterize the schizophrenic, and maybe within the next few years we may be able to say to you that, for example, the schizophrenic's corticosteroids consist of compounds E, F, G, U, R, and V, whereas non-schizophrenics have another group, but until that day of specific steroid characterization comes I don't think that question can be answered.

ZUCKERMAN: You have given precisely the answer which I thought might emerge. We *have* got an extremely interesting series of observations, which at the present moment cannot be properly articulated into a single pattern.

REISS: A very important question has been touched here: the causal relationship between the function of certain ductless glands and psychosis. Two possibilities have to be borne in mind: certain mental disturbances can most probably be caused by disturbances of the adrenal cortex function; but it is also known that under the influence of psychic

bodily comfort or safety, it will give rise to widespread reaction. Simple nervous reflex, in contrast, is instantaneous, and when not controlled by the brain, like a penny in the slot, gives the same reaction. However, simple reflexes [illegible] functions [illegible] when so [illegible] dictable. Repetition, therefore, forms the basis of reflex education. It is not until we reach the hypothalamus, the junction of the vegetative and spinal reflex systems, with their centres of rage and fear, that we come to the emotional centres.

The expression of an emotion, like instinct, is innate. It does not require to be learnt, it is not entirely controlled by the will, and is in the nature [illegible] The forebrain, therefore, [illegible] heritage; it may inhibit and rationalise, but fundamentally, it cannot always master the sudden accidents of environment, or the promptings of its instinctive and biochemical processes.

From a long clinical experience, I may be permitted to make a few general remarks on the material which has presented itself to our out-patient department.

The first is that psychosexual disorders predominate in the female sex, who are more unstable than the male. Our observations upon what may be regarded as a short and transient male phase in the female embryo, would bear this out. My second impression is that the generally accepted view of being able to condition a patient to his environment is a hopeless task if his physical make-up is maladjusted towards it, or if there is some powerful outside cause, which cannot be removed. With regard to the clinical material, this may be classified under three broad headings:—

(1) Psychosexual abnormality without any obvious endocrine disease.

(2) Indefinite or abnormal psychosexual behaviour in association with *varying grades of intersexuality*. In this group the psyche and the soma become confused and bear no definite relation to each other.

CLINICAL TYPES OF ALTERED SEXUALITY IN RELATION TO ADRENALECTOMY

L. R. BROSTER

From my earliest experiences in adrenalectomy, dating from 1926, for virilism due to adrenal hyperplasia, I became aware that some patients presented abnormal psychological reactions, which reverted to normal after operation. We may safely accept this as an established clinical fact. We were obviously dealing with a psycho-somatic problem, and the only evidence we have since been able to supply is that the ketosteroid excretion in these cases is altered as a result of operation. As our experience increased and our work became more widely known, we became confronted with a bewildering assortment of psycho-somatic disorders, and it was for this reason that Dr. Clifford Allen, who was then psychological assistant to Charing Cross Hospital, became associated with this work. I therefore leave him to deal later with the problem from the psychological point of view. For my part, I can only speak as a general surgeon, a clinician, tried in the hard school of practical experience, and my contribution will be mainly from the somatic point of view. Together we have published cases, which we have considered typical of the studies we have undertaken.

In the first place, it is necessary to make a few general remarks on the evolution of behaviour, even at the cost of simplification and generalisation. All behaviour, however complicated it has become, is based on instinct. This, Trotter defined as " *the inherited modes of reaction to bodily needs and external stimulus* ". In the lowest animals, instinct is spontaneous and largely an expression of biochemical reaction. Upon this primitive form of chemical control has been superimposed a nervous system of ever-increasing complexity and widening reactions. Simple instinct, though akin to reflex nervous action, is delayed, complex, and influences the body as a whole. *It is innate*

bodily comfort or safety, it will give rise to widespread reaction. Simple nervous reflex, in contrast, is instantaneous, and when not controlled by the brain, like a penny in the slot, gives the same reaction. However, simple reflexes combine to form groups which perform the ordinary functions of the body, and when conditioned, form habits, and when so linked, their reactions are likely to become unpredictable. Repetition, therefore, forms the basis of reflex education. It is not until we reach the hypothalamus, the junction of the vegetative and spinal reflex systems, with their centres of rage and fear, that we come to the emotional centres.

The expression of an emotion, like instinct, is innate. It does not require to be learnt, it is not entirely controlled by the will, and is in the nature of a widespread reflex discharge. The forebrain, therefore, cannot escape from its animal heritage; it may inhibit and rationalise, but fundamentally, it cannot always master the sudden accidents of environment, or the promptings of its instinctive and biochemical processes.

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other girls. She begins to shun society, becomes frigid, careless about her dress and appearance, and avoids the society of young men. She is obviously trying to escape from her social environment. At this stage she tends to become emotional and afraid. Her future is at stake, her chances of matrimony are receding, and to earn a living in public becomes distressing to her. She now becomes a serious problem to her friends and relations. When this stage has been reached, one of two alternatives may happen, which suggest that the higher faculties are involved.

She may remain predominantly feminine and live the life of a hermit. Sooner or later delusions will appear. She will begin to imagine all sorts of things, that people are whispering or saying things, that they are plotting against her, or that the police are after her. She may imagine that she is unclean and smells, or that she must urinate like a man, standing up.

Or, on the other hand, she may find that her acquired male characteristics give her a sense of power or ascendency over her female friends, and she becomes frankly homosexual. Indeed, some of these women boast that they can outrival any male in the art of courtship.

From this short summary it is suggested that behaviour forms part of a biological process that has become disorderly, and that psychological reaction, like structure and function, has climbed up its own genealogical tree.

Let us now consider what evidence there is which has a general bearing on the problem In the first case, genetic determination may not run true to type, and there is some evidence to suggest that it may be over-ridden and sex determined by endocrine means This endocrine control may remain latent, and exert its control any time during life. Complete sex reversal, which is only partial in man, occurs in lower animals. In fowls which we have studied, there is usually present a colloid carcinoma of the ovary. In the freemartin of cattle, we have a similar effect, where the female co-twin becomes masculinised by the male. It is also worth mentioning that when the adrenals fail to shrink in size at birth, there is a marked lack of development of the cerebral cortex, resulting in anencephaly The most

(3) Subnormal, autosexual or abnormal behaviour in association with the adrenogenital syndrome.

The clinical evidence of a powerful external stimulus as a cause of somatic changes is meagre. It is suggested in two types, differing in girls and boys. For instance, the young girl who is acutely unhappy at home. She will develop hair of the downy type, and her menses will become irregular or cease. She may go on hunger strike and develop into a typical case of anorexia nervosa. These patients recover once the source of unhappiness is removed. The second type, usually the only son, the so-called Fröhlich's syndrome, mother's fat, rosy-cheeked, pampered darling sugar baby, often mentally backward and sexually undeveloped. These boys will improve on hormone therapy.

The evidence of an abnormal soma associated with psycho-sexual disorder is more convincing, and has occurred in about 20 per cent of our cases suffering from the adrenogenital syndrome which have undergone unilateral adrenalectomy.

In the adrenogenital syndrome, the physical changes depend on the time of onset. All these patients are brought up as girls. In the prepubertal group, they do not develop their secondary female sex characters at this time; consequently, they are diagnosed and treated early. Their sexuality is ill-defined, or may be neuter, which is in contrast to the children with isosexual precocity of adrenal origin, where the sexuality may be strongly precocious. In two of our patients who had reached adult age, their behaviour had been bisexual, first male, and later female. These patients excrete androgens in large amounts.

Where the syndrome arises after normal puberty and sex development, the sexuality is more insidious. The usual history of these patients is as follows: Hair of the male type and distribution begins to appear. This has a powerful psychological effect on young women in the form of an instinctive repugnance to the unjust imposition of an atavistic character. She becomes shy, self-conscious, and depressed, and may develop a marked inferiority complex. The second symptom to appear is an irregularity or cessation of the menstrual function. Sooner or later she begins to correlate this with her hirsuties, and to think that she is different from

was isolated from our assays pregnanetriol, a substance specific to virilism, which yielded *iso*androsterone among its first derivatives. In our long series, the amount of androgens was considerably reduced by means of the operation of unilateral adrenalectomy.

The clinical and surgical results may be tabulated as follows, up to 1946:

Table II

Adreno-genital syndrome	Keto-steroid results	
	Pre-	Post-operation
Average 41 tests	17.6	8.5
Good result	17.5	6 8
Improved	19 1	9 9
I S.Q.	15 1	8.4

Average: age 26, observed 16 years. Mortality, nil.

Table III

All cases	Virilism	Cushings	Psychological
All cases	88	14	15
Good result	16	2	5
Improved	45	11	8
I S Q.	10	1	2
Deficient information	14	—	—
Died other causes	3	—	—

It will be seen that the ketosteroid excretion has been reduced by about 50 per cent, and the best results are those in which the post-operative excretion is lowest, and over 50 per cent. The pre-operative ketosteroid figures in the psychological patients are usually not high, and in some abnormal psychology may be the main indication for operation. It is, therefore, impossible to say which cases will improve by operation, and the failures have mainly been in those cases where there is an external cause which cannot be removed. These results are entirely empirical, and it is not known how the mechanism acts.

important observation was made by Vines. By means of the Ponceau-Fuchsin stain, he was able to show that the cells of the adrenal cortex, in the adrenogenital syndrome, took up the vivid red Fuchsin stain, in contrast to the methylene blue counter stain of normal controls. On applying this stain to fœtal adrenals, it was also found that it was present in the embryos of both sexes for a transient period roughly between the 8th and 18th week. Every female, therefore, passes through a male phase, which may be prolonged or reappear in later life (Table I).

Table I

FŒTAL TIME-TABLE

Weeks	
5½	Indifferent sex-glands.
6	Testes and adrenals appear
6– 8	Ovary. Utero-vaginal canal commences.
9	Interstitial cells of testis.
	Sex differentiated structurally.
	End of chromosomal phase.
9–17	Fuchsin stain in male adrenal cortex.
11–14	Fuchsin stain in female adrenal cortex.
12–14	Pituitary gland.
13–15	Acidophil and basophil cells.
15	Fuchsin disappears from female.
12–16	Utero-vaginal canal separated.

The adrenal cortex may, therefore, be said to play the part of an accessory male sex gland. It will also be seen, from this timetable of fœtal integration, that starting about the 5th fœtal week, the ovary and then the testis appear just before the adrenal goes through its male phase, which ends about the same time that the pituitary becomes differentiated. It is also during this time that the chromosomal phase ends, and may be materially altered by the pattern of endocrine integration.

The presence of the Fuchsin stain suggested that the cells of the adrenal cortex were secreting male hormone or prohormone. *Urinary assays by Prof. J. Patterson showed that* they produced comb growth in capons on the old biological test, and later, when the ketosteroid test was developed, there

THE RELATIONSHIP OF STEROIDS TO PSYCHOSIS

CLIFFORD ALLEN

THE first observers who noticed psychical changes connected with the administration of cortisone or ACTH, were Hench (1949), and his colleagues. They commented on the "euphoria" which seemed to follow it. The hormones had been given for the relief of rheumatoid arthritis, and the
nted on as a side effect.
response, and discussed
pain or whether it is a
genuine euphoria. They thought that it was a genuine euphoria and two of this team (Braceland and Rome) suggested that it might be a substance beneficial in the treatment of mental disease

However, other observers have noticed that some patients after cortisone or ACTH go further than merely feeling euphoric, and develop a true psychosis. This has been discussed by Shackler (1950), Thorn (1950), and others. Altschule (1950), describes a case and says: "In recent months it has become clear that the injection of large amounts of ACTH or of cortisone (17-hydroxy-11-dehydrocorticosterone) may give rise to mental changes in persons previously normal. More than two years ago, before this was known to us, certain considerations led to the use of ACTH in large amounts with two patients. The first, a woman aged 66, with manic-depressive psychosis, depressed type, received 570 mg. in 17 days; after a few days she became more active, and this change was deemed improvement. A few days later, however, hallucinations developed. At the end of the 18 days period of injection, she was still extremely agitated and
ocks
the
the

It is interesting to note that a fall in ketosteroid output has been reported after the operation of pre-frontal leucotomy. This relation between disordered mental states and androgen metabolism is an intriguing problem. It offers an avenue of research which, in our present knowledge, suggests that psychological problems may be interpreted partly by biochemical means. This argument is not necessarily disproved by quoting cases of genetic homosexuality, where no endocrine factor exists. It seems likely that there may be two types, one genetic and one endocrine; just in the same way that Cushing's syndrome may exist either as pituitary basophilism, or adreno-cortical manifestation; or iso-sexual precocity from hypothalamic tumour or adrenocortical carcinoma.

This case is interesting, since it shows the onset with agitation and press of activity which prevented her sleeping. This might easily be mistaken for euphoria by those who were not expecting it. Then the patient became more depressed, as did Altschule's case, and finally grossly hallucinated. The hallucinations do not seem to have been abusive, nor do they throw any light on the patient's sexuality, but this may be due to the fact that she is unreliable and conceals things. She did at first conceal from me that the hallucinations were still persisting nearly 3 months after the injections had ceased.

The appearance first of depression, then agitation, and finally hallucinations, is most interesting, since that is exactly what we have found in the adrenogenital psychoses. Milder ones have been depressed and, in all cases, felt " brighter ", after adrenalectomy. The more severe ones, however, were hallucinated but not deluded. They had fugacious delusions when they were at the zenith of their psychosis.

The object of this paper is to suggest that there is a definite relationship between psychosis and disease of the adrenals. In fact, there seems to be an entity the " adrenal psychosis." if we use the term in a similar way as we use " alcohol " in alcoholic psychosis. No other gland is so related to psychosis as the adrenal. It seems quite evident that psychoses are associated with adrenal hyperplasia, and this was first pointed out by the Charing Cross team as early as 1938, in our book " The Adrenal Cortex and Intersexuality (1938) " (page 73), where it was stated " Two adrenogenital cases suffered from depression and two suffered from schizophrenia". I formed the opinion that the milder cases showed depression and the more severe ones schizophrenia.

This view that the psychoses are associated with adrenogenital virilism, has been confirmed by Soffer (1948), who is apparently unaware of our work, since he makes no mention of it, in his book on " Diseases of the Adrenals." He states : " The patients frequently manifest *severe depression*, occasionally *mania and excitement*, and *periods of paranoid confusion*. They are frequently preoccupied with *thoughts of suicide*, which constitutes no idle threat, and which requires careful watching. However, depression is most commonly

injection of the ACTH. The second case merely showed worsening of the depression from which she suffered.

Through the kindness of Mr. Broster, I have recently seen a case of a woman who had had an adrenalectomy some 16 years ago for virilism. I had seen her then and noted that she seemed sexually normal, but was an unstable neurotic type. She remained more or less well until 3 years ago when she was aged 38 years. Her mother then died and this upset her a great deal. She developed fever and arthritis soon afterwards, though whether this was connected with her bereavement one cannot say. Her illness made her take to her bed and she could not walk. This was diagnosed as rheumatoid arthritis, and various treatments were given without effect. Finally, her husband was able to obtain some cortisone, and this was administered. She had been a difficult patient before she had the injections and was started on 25 mg. daily. This produced little clinical effect, and the dose was increased to 100 mg. per diem, with slight improvement. As her mental condition did not seem affected, she was given 200 mg. for one day. Seven hours after the injection, she became excitable, agitated, talked continually and could not sleep There was depression and constant weeping, which it was beyond her volition to stop. Then she felt that people were spying on her, and heard "voices." Very expressively she says that "the very walls themselves seemed to be talking." There were no visual hallucinations. The voices mainly echoed her thoughts or made comments on her behaviour. The dose was reduced to 100 mg. and then raised again to 150 mg. With this dosage on some evenings she had delusions that there were people in the corner of the room. Although she was depressed in the day-time, at night she became wildly excited. Then she had sweating, rapid pulse, over-breathing and fluttering of the eyelids. The cortisone was discontinued for 2 days and then re-commenced with 25 mg. and finally 50 mg. as a maintenance dose. The total dosage given between 20th December, 1950, and the 13th January, 1951, was 2 gm. She still (13.3.51) has some occasional hallucinations, although these are now rare and she has proper insight that they are psychogenic.

to have been examined by a psychiatrist, but which presents similarities to our own, and I feel she was really a paranoid psychotic. She showed hirsutism, hypertension, osteoporosis, abdominal striæ, and a diabetic Janney curve. "In addition, she was quarrelsome and unco-operative. She manifested marked persecutory trends, and regarded the other occupants of the ward and the medical attendants with hostile suspicion. She had periods of depression during which she would neither eat nor talk. Quite suddenly the depression would lift, and be followed by a phase of relative excitement. On several occasions she threatened suicide, although no actual attempt was ever made. After successful removal of an adenoma of the right adrenal, there occurred a gradual recession of both the physical and mental symptoms."

It would be a miracle if all cases responded so successfully as these, and we know they do not do so, but it is hoped enough has been said to show there is a most intimate relationship between the adrenal and these psychoses. If we accept that there is such a thing as an adrenogenital psychosis or a cortisone psychosis (or if they are the same thing and we call them an adrenal psychosis), and it seems impossible to contradict that they do occur, if we accept this, how are we to explain that all those suffering from adrenogenital symptoms do not develop a psychosis? Why is it that only infrequently those given cortisone become psychotic?

No definite answer can be given, and anything suggested must be speculative. However, there seem to be two possibilities. Firstly, they could be an ordinary toxic psychosis such as we find follows the use of alcohol or other drugs. This, I think, is disproved by the nature of the psychoses. Firstly, in those I have seen, there have been no physical signs of toxæmia: no coated tongue, hot dry skin, dehydration, picking of the bedclothes and so on. Moreover, the characteristic of toxæmia is usually *visual hallucinations*, and these seem never to occur. There are none of the streaks of light, rats, mice, or insects, and so on, of the alcoholic, nor the disorientation of person, place, or time so usual in toxic delirium. If the adrenal psychoses are toxic, they present a type of toxæmia not previously recorded in psychiatry.

noted, and almost all patients with adrenal tumours develop this symptom to varying degrees. *Irritability* is not infrequently observed, and the *quarrelsome tendencies* may constitute a ward problem."*

This is more or less our own experience, except that mania is unusual, but we have seen a case in which mania appeared *after* adrenalectomy and formed a very difficult problem of nursing.

It might be argued that the psychoses which we have seen were purely fortuitous, or else due to the distressing hirsutism. This is discounted by the fact that in our first series we had 4 cases in 37, which is much above the number in the normal population, and, moreover, their response to treatment is curious.

After the publication of our book, the late Dr. R. D Gillespie very kindly sent to us a case of paranoid psychosis in a woman suffering from adrenogenital virilism. (This case will not be described in full, nor will the following one, since they have both been published.) However, the result of adrenalectomy was brilliant. The woman lost her hallucinations and delusions within a month. This, I think, can be claimed as the first case of adrenal psychosis which was deliberately cured by the removal of an adrenal gland. We published this case in full in 1939. She has remained well until recently, when during her menopause, she had a slight recurrence of her psychotic symptoms.

Fortunately, we were able to discover a further case, and this responded just as well as the previous one. We published this in 1945, but the only result of these two publications was the raising of politely incredulous eyebrows. Dr. Raymond Greene and his colleagues (1945), published a case of depression, which responded similarly to adrenalectomy.

Broster, in a personal communication, states that he has had a further case of psychosis in adrenogenital virilism. He removed the adrenal and recovery took place and has persisted. Owing to the attitude of the psychiatrist in whose care she was, the writer did not see this patient.

Soffer (1948), records a further case which does not seem

* His italics throughout.

adrenogenital psychosis patient produced notes which showed that she had homosexual interests, although the idea of being like a male horrified her. The second patient was very masculine in behaviour, and wore "slacks" and smoked incessantly. After adrenalectomy, she preferred dresses. A patient with depression whom I have under my care at present, is consciously homosexual, and has fallen in love with other women. In her case, adrenalectomy produced no benefit. All this must be somewhat speculative, but it is difficult to find any other theory which offers any explanation from the psychiatric point of view. Thorn says, I believe truly: "The mental changes associated with ACTH and cortisone therapy, have been varied, but, in general, have appeared to represent distortions in the patient's pre-treatment personality." In other words, the steroids only release the inhibitions on what is already there.

To sum up: psychoses are associated unduly frequently with diseases of the adrenal gland. In adrenogenital virilism, they are sometimes curable by adrenalectomy. They appear to be caused by interference with inhibition.

Note.—I am grateful to Dr. Kersley of the National Hospital, Bath, for information regarding the cortisone patient's psychosis in its acute stages.

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DISCUSSION

ZUCKERMAN. A long time ago one occasionally used to read of the feminization of males due to abnormal adrenal function

BROSTER Yes, we had a feminized male, who became feminized after puberty. His ketosteroids were about an average of 8 6, pre-operative. We removed his adrenal—he had a definite fuchsin stain—

The second possibility is that the steroids interfere with inhibition and allow patterns of behaviour to emerge which would not have done so otherwise. Psychoses are diseases of inhibition (hallucinations, for example, are probably only thought-processes which should have been inhibited). It has long been the belief of some psychiatrists that the psychosexual responses of psychotics are abnormal—even when no endocrine or other physical cause can be found. Connor (1948), found 90 per cent of suicidal depressions showed an unsatisfactory sexual life either in the weakness of the heterosexual urge or the strength of the homosexual urge, or both. Pavlov has shown that a psychical disturbance, neurosis or psychosis, can be produced by conflict of negative and positive excitations. If the normal heterosexual urge is reduced, and the homosexual patterns which are normally inhibited are released, such a conflict could be induced.

We found in our first series of adrenogenital virilism cases, that out of 8 homosexuals, 4 were changed to heterosexual, and 2 were doubtful after adrenalectomy. One was unchanged. Of 16 heterosexuals, an increased heterosexuality was noticed in half, after the adrenal was removed. We can take it, therefore, that the 17-ketosteroids in adrenogenital virilism indicate depression of the normal sexuality, and thus reflect the conditions we would postulate to produce a psychosis. Adrenalectomy, or the cessation of the cortisone injections, allows the brain to subside into its previous equilibrium, and the symptoms to disappear, but this does not happen immediately, and the symptoms may persist for some time, and even not be cured. We have seen two or three cases in which adrenalectomy has not cured a psychosis—I have one case of depression in my hands at the present moment in which adrenalectomy had no effect, and another case of schizophrenia which received no benefit. In these presumably, the removal of the adrenal was evidently not enough, because the psychical elements were too strong.

If this is the case, then one might expect to find signs of homosexuality, though perhaps only minor ones, in the behaviour either before or during the psychosis. Does this occur?

It certainly does in some cases: for example, our first

them 48 hours after adrenalectomy that their hair comes out fairly easily by depilation. It seems to act directly on the hair roots. And if their menstrual function becomes normal afterwards, they receive their second stimulus and it helps enormously in adjusting their psychology.

There is one point I didn't mention. A number of these women are frigid, and one of the results of adrenalectomy is to increase the fertility. We have taken biopsies of ovaries, and nearly all the ovaries are cystic and degenerate, so that the adrenal seems to have a depressive effect on the ovary, and probably that accounts for the decreased function.

ZUCKERMAN Dr Beach's main charge does not appear to have been met, in so far as you have not disposed of the possibility that the women might have become better if they had used a hair remover instead of having their adrenals removed But you say that women will not shave, that they never use razors—so that disposes of this issue for the moment. Dr. Beach has, however, also suggested that your criteria for abnormal behaviour are somewhat tenuous.

ALLEN. My criterion of homosexuality is falling in love with a person of the same sex, if someone was really attracted so strongly as that then I would say he was homosexual. He may show no overt behaviour at all, it may be contrary to his ethical feelings

BEACH· What about schoolgirl crushes?

ALLEN. They are passing through a homosexual phase, presumably through some disturbance in their glands. I don't know enough about the disturbance of puberty on the mind; I don't know why they pass through a homosexual phase, but girls do, and boys do [illegible]

PINCUS· There are now a large number of clinical cases in which steroids have been administered: cases in which women have received

FOLLEY· There was a paper in last week's *British Medical Journal* by G L. Foss of Bristol, describing a number of cases showing that administration of androgen had increased the female libido.

ZUCKERMAN But these women presumably knew that they were being slightly transformed in a masculine direction, and might, let us say, have tried to intensify their feminine drives in compensation.

and we reduced his ketosteroids down to about six. He was 32 years of age, but he grew an inch; he put on a stone in weight; his sexuality improved—he hadn't any before—his beard grew and his voice dropped somewhat; his genitalia enlarged.

ZUCKERMAN: Have any other similar cases been reported?

BROSTER: No. I had another case like that, but he wouldn't agree to have adrenalectomy done.

ALLEN: We did have a schizophrenic psychotic. He was excessively hairy, but there was no feminization.

BROSTER: A fat, very hairy male may certainly show feminine characters, and generally has tender nodules in the epididymes. Excessive hair in the male seems to be a feminine character, and is probably due to secondary endocrine imbalance.

CLEGHORN: I would like to take issue on that It seems to me that a hair distribution one has to consider the racial factor. One has to

[illegible]

attitude from Sandinavian races, for instance, which are apt to be more hirsute. To judge a person as feminine on the basis of the amount of hair on his chest is fallacious.

BEACH: We need a more explicit definition of basic terms. To imply that a woman who wears slacks and "smokes like a chimney" is therefore homosexual seems hardly justifiable. But even if we restrict our dis- [illegible] that physio-

[illegible]

[illegible] they will not use a razor

[illegible]

œstrous female will mount another female, and not only mount but actually show pelvic oscillations as though she were a male, and that in turn she will be mounted by the œstrous or non-œstrous female, often exhibiting exactly the same masculine pattern, quite independently of her sexual condition. It is a most common phenomenon. The male pattern of behaviour of mounting and so on is latent in the female and may appear in any situation in which there is sexual excitement, without adequate opportunity for heterosexual contacts.

Allen I am glad to hear you say that, because I treat a large number of homosexuals as patients. I have seen perhaps 200 of them, and it seems to me that in human beings homosexuality is also a social problem to some extent, only the social problem starts in the child. But this

homosexuality.

Walton Is psychosis then an effect of isolating the individual from his normal heterosexual contacts, so that he finds sexual expression in the field in which he is isolated?

Allen Not in the psychoses produced by cortisone, for example, because the injection of cortisone produces it; there is no isolation there.

Zuckerman: Can we be quite clear as to what are the divergent views here? As I understand it, Dr Allen is propounding the idea that homosexual behaviour is definitely dependent on some mal-functioning of a specific tissue. Is that correct?

Allen. Yes, I think the normal sexuality is depressed We did find in our series of cases that people were more heterosexual after adrenalectomy than they were before.

there may be a reversal of behaviour.

Allen: I think the normal behaviour may be suppressed and behaviour which had not come out before, will come out.

Folley There is a condition in cows that Dr. Walton did not mention It is well known that cows suffering from nymphomania

BEACH This also occurs in animals. If one treats spayed female rats with testosterone propionate, they show a low degree of œstrous behaviour, a recognizable change from the castrate condition.

ZUCKERMAN: But do you provoke œstrus in a normal rat with testosterone?

BEACH: I don't know.

BROSTER. I am now treating a woman for carcinoma of the breast with a huge implant of testosterone. I have removed both her breasts for carcinoma, and she now has secondaries in the spine. Since the implantation of testosterone there has been an increase in the growth of hair and masculine symptoms. Her voice is deeper, and she is

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such a good second honeymoon, that he thought we had overdone it.

ALLEN· Isn't is true that if you give psychic homosexuals testosterone, that they become more homosexual?

PINCUS Yes.

ALLEN· Surely that shows that the effect is dependent upon the psychology, to some extent, at any rate

PINCUS That is a point in which I am interested. This is an increase of the original drive, perhaps, with no reversal of psychosexual behaviour. My understanding was that when you performed adrenalectomy on a woman who had been homosexual, she became more heterosexual, and I want to know whether this is due to removal of androgen or to something else?

ALLEN: I did say that this was only speculation. One might imagine it to be a balance between heterosexuality and homosexuality.

WALTON. I think my experience with animals bears out entirely Dr. Beach's postulation that homosexuality is primarily a social phenomenon. One can produce homosexuality in animals very easily. For instance, our method of obtaining semen samples is to put an incentive animal in a crate and then lead the male up to it. The male mounts the incentive animal and attempts copulation. We collect semen by interposing an artificial vagina into which he ejaculates. Males will

thing occurs with males kept together but isolated from females; they will mount each other and perform homosexually. Exactly the same thing happens with females. If you have a group of sexually active females together and one or more come on heat, you will find that the

œstrous female will mount another female, and not only mount but actually show pelvic oscillations as though she were a male, and that in turn she will be mounted by the œstrous or non-œstrous female, often exhibiting exactly the same masculine pattern, quite independently of her sexual condition. It is a most common phenomenon The male pattern of behaviour of mounting and so on is latent in the female and may appear in any situation in which there is sexual excitement, without adequate opportunity for heterosexual contacts.

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ALLEN· Yes, I think the normal sexuality is depressed We did find in our series of cases that people were more heterosexual after adrenalectomy than they were before.

ZUCKERMAN And the contrary view, which Dr. Pincus has emphasized and which many others here share, is that if you charge an organism with more hormone, say androgen, all you do is intensify behaviour previously manifested You, Dr. Allen, on the contrary, indicate that there may be a reversal of behaviour

ALLEN I think the normal behaviour may be suppressed and behaviour which had not come out before, will come out

FOLLEY There is a condition in cows that Dr. Walton did not mention *It is well known that cows suffering from nymphomania* (presumably due to excessive production of œstrogens) can behave like bulls, not only in the situation of mounting, but they actually come to look rather like bulls They bellow in the characteristic male way, paw the ground and behave rather like bulls. We have seen the same thing in experiments on the artificial induction of lactation where normal cows are being given fairly high dosages of œstrogen over long periods, they show the same apparent reversal in sex behaviour.

BEACH: This also occurs in animals. If one treats spayed female rats with testosterone propionate, they show a low degree of œstrous behaviour, a recognizable change from the castrate condition.

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PART III

PSYCHOLOGICAL AND BEHAVIOURAL REACTIONS AS SIDE EFFECTS OF STEROID ADMINISTRATION

ALTERATIONS IN PSYCHOLOGICAL STATES BY THERAPEUTIC INCREASE IN ADRENAL CORTICAL HORMONES

R. A. CLEGHORN

REPORTS describing abnormal psychological states in patients with adrenal cortical hyperfunction have appeared with increasing frequency in recent years (Holmes, 1925; Broster and Vines, 1933; Broster, 1938; Maclay *et al.*, 1938; Allen and Broster, 1945; Greene *et al.*, 1945; Broster, 1946, Soffer, 1946). Not all such cases of hyperfunction show mental symptoms, and in fact the incidence is not known, as this type of aberration is often but incidentally described, as part of the complicated clinical and metabolic changes which have been the chief preoccupation of most observers. There does, however, seem to be a similarity in the psychopathology of the mentally disturbed cases (Soffer, 1946; Glaser, 1950). It has been described as resembling paranoid schizophrenia.

The explanation of the mental symptoms, tacitly implied if not always expressed, was that this was an adverse psychological reaction to cosmetic and other disfiguration, in an unstable personality. This was not applicable in one of Soffer's cases at least, for there the psychological symptoms preceded the physical. Furthermore, in certain cases subjected to unilateral adrenalectomy, psychological improvement seemed to precede major changes in the physical state.

BROWNE: I would like to point out that I accept entirely what Mr. Broster has said on the effect of adrenalectomy, and I would like to point out certain things which also can occur, and will report two cases to illustrate. The first was a woman who was very definitely virilized, with clitoral hypertrophy and baldness, and because it was thought that perhaps her ovaries might be involved (she was 56 at this time) the operation was hysterectomy and removal of the ovaries. A careful [illegible]

frequently as she previously did, for about three months Then her ketosteroids rose again, and she had to return to shaving once a day. The second case was one of hirsutism. An attempt was made to operate upon her for removal of part of the adrenals, but they never reached the adrenals because she had such a severe hæmorrhage during the course of the operation. All her excess face hair fell out after the [illegible]

become cachectic, as one would have expected in a malignant tumour with this degree of metastases, so the androgens may have kept him together physically until he died of a gastro-intestinal hæmorrhage.

Thirdly, a case of Cushing's syndrome, a woman, to whom methyltestosterone was administered. Under methyltestosterone her ketosteroids fell from about 60 mg./24 hr to about 16 mg /24 hr., and she began to menstruate. She then became pregnant.

and Baehr (1941). Multiple grand mal seizures were seen in one of eight depressed patients being treated with ACTH (Cleghorn *et al.*, 1950). Recovery occurred.

The personality changes occurring under the influence of these agents may be very diverse. Rome and Braceland (1950), say that " the personality conflicts of psychologically inadequate patients--fulminated, resulting in hypomania, mild depression or both, and sometimes in ritualistic behaviour. Rarely a psychosis of brief duration was precipitated." The mental upset was severe enough in two cases of Pearson and Eliel (1950), to be associated with suicide. Kuzell and Schaffarzick (1950), said psychosis occurring with cortisone was transitory. Klein and Livingstone (1950), found "severe psychic disturbances" in epileptics given ACTH. Hench (1950), opines that major psychotic changes, when they occur, are mainly in persons with preexisting psychotic personalities. This may be true, but case 11 of Hoefer and Glaser (1950*b*), is said to have had schizoid personality features prior to treatment, and yet developed no [illegible] Another of their patients (Case [illegible] een ACTH and cortisone on t[illegible] the former, he was more te[illegible] and mentally hyperactive, on the latter less so.

Hoefer and Glaser (1950*b*), report significant E.E.G. changes in 13 of their 15 cases, a slowing in rate on the whole. They correlate this with alterations in personality in 10 of their 15 cases. E.E.G. changes were not noted in our ACTH treated cases (Cleghorn *et al.*, 1950).

Ransohoff *et al.*, (1950), reported in considerable detail the development and alleviation of a psychosis during ACTH therapy. She was an anxious, easily disturbed woman with many neurotic traits On the 43rd day of ACTH therapy, a gross paranoid psychosis developed. This was associated with the appearance of slow activity in the E.E.G., and a severely low serum K level Given 6 grams of KCl orally, she ceased being psychotic in 12 hours, and the E.E.G. became normal. She again became psychotic, and the E.E.G. abnormal, and cleared up again with 400 ml. 2 per cent KCl intravenously over 3 hours. This well documented

In view of the clinical evidence of the association of psychotic states with adre
hypofunction, it is not now
and cortisone caused psyc
cases reported. The record of mental changes in patients treated with ACTH or cortisone has been by incidental observations largely, side issues in reports devoted to the lavish description of striking biochemical changes and still more startling clinical improvement of many diseases hitherto resistant to therapeutic efforts. At present, there is a paucity of detail concerning the mental changes and the patients who show them.

Positive mood changes were early observed in patients with rheumatoid arthritis receiving ACTH or cortisone (Hench *et al.*, 1949; Sprague *et al.*, 1950; Hoefer and Glaser, 1950; Hench *et al.*, 1950; Freyberg, 1950; Spies and Stone, 1950). The readiest explanation is that the sudden relief of a painful and crippling disease is enough to make the most morose euphoric. There is evidence that this is not the correct explanation. In the first place, Hench *et al.*, (1950), say that their patients on ACTH are less euphoric than those on cortisone. Furthermore, other patients with less obviously crippling and less rapidly responding lesions became euphoric, e.g., in pneumonia (Finland *et al.*, 1950; Kass *et al.*, 1950), ulcerative colitis (Du Toit and Bauer, 1950), and cancer (Taylor and Morris, 1950)

In some instances, the elation early in the course of treatment went on to hypomania, and even manic states (Hoefer and Glaser, 1950; Du Toit and Bauer, 1950), requiring electroconvulsive therapy (ECT).

Negative mood changes have also been produced by ACTH and cortisone, and the resulting depression can be quite profound (Freyberg, 1950; Rome and Braceland, 1950; Soffer *et al*, 1950; Hœfer and Glaser, 1950; Reeder and Mackey, 1950; Cleghorn *et al.*, 1951).

Convulsions as a complication was seen in one case by Elkington *et al.*, (1949). In acute disseminated lupus erythematosis it was seen in four instances by Soffer *et al.*, (1950), one of whom died. Convulsions are not an infrequent occurrence in this disease according to Klemperer, Pollack

(Cleghorn *et al.*, 1950). This negative finding is in accord with observations of Rome and Braceland (1950), on 3 cases with depressive psychosis of the involutional period.

(b) *Schizophrenia.*—The work of Pincus and Hoagland (1950), would lead one to hope that this disease might respond to ACTH therapy. Cases so far treated by these authors, 2 cases by Hœfer and Glaser (1950*b*), 3 by Rome and Braceland (1950), have not benefited significantly.

(c) *Involutional Melancholia.*—As long ago as 1942, Hemphill and Reiss (1942), treated some cases of involutional melancholia with ACTH with alleged success. Recently, Spies *et al.*, (1949), reported good results in one such case.

Effect of Restoration of Deficiency of Adrenal Cortical Secretion

1.—*Treatment of Hypopituitarism*

In all the above record, treatment has presumably led to an excess amount of cortical hormones What happens when a deficiency is corrected by these new and powerful agents ?

The case of hypopituitarism described by Schrock *et al.*, (1951), was already hallucinatory when treatment was started with ACTH, as well as being lethargic. This 46-year-old woman was also receiving lipoadrenal extract and testosterone, which were continued, the former for 4 days, the latter for 80 days. The immediate effect of ACTH in the first 6 days of its administration was to increase hallucinations and induce agitation which required restraint. Meanwhile, the blood sodium rose from about 290 to 320 mg. per cent, and the serum K fell from 20 to 15 mg. per cent. After a few more days, the psychotic behaviour became less pronounced. The ACTH was interrupted after about 10 days, and after about 14 more days, cortisone was begun, and it was noted that this was accompanied by extreme modesty, and growth of axillary and pubic hair. The rheumatoid arthritis from which she was suffering also improved.

2.—*Treatment of Addison's Disease*

A patient with Addison's disease under the care of Dr. J. S. L. Browne revealed a somewhat similar response to

study will serve as both direction and stimulus to further work.

A girl of 19 suffering from dermatomyositis and muscular dystrophy, was admitted to the care of Dr. Donald McEachern at the Montreal Neurological Institute, and was treated with cortisone. He has kindly permitted me to cite her case, which is better documented than most so far reported. After receiving 100 mg. cortisone daily for 28 days, the patient suddenly became incontinent and withdrawn. Cortisone was stopped, but the negativistic behaviour progressed. She stared straight ahead, and would neither eat nor talk. An E.E.G. taken 3 days after the onset of this episode was abnormal, showing low voltage 18 to 20 per sec. waves of 10 to 20 m.v. all over the head, as in tension or anxiety states. The serum sodium level was 132 M. eq/l and potassium 3.9 M.eq/l. Under amytal, the patient opened up and talked and revealed some incest guilt with respect to her twin brother, and she felt that her sickness was a sexual disease. She also expressed sorrow for trouble she was causing. A few hours later she was withdrawn again, and continued in her catatonic behaviour. ACTH 25 mg. × 4 for a few days was ineffectual. Testosterone 50 mg. daily × 3 was of no help. Nasal feedings were resorted to.

On 22nd December, after 27 days withdrawal and no improvement, E.C.T. was instituted. After the second, she was somewhat improved, and by the fourth she was approximately normal. Withdrawal soon occurred, so E.C.T. was continued, and 26 treatments in all given. The final diagnosis was catatonic schizophrenia.

Therapeutic Trial of ACTH in Psychiatric Cases

(a) *Depression.*—On the basis of the early reports of euphoria and the evidence that E.C.T. activates the adrenal cortex (Hemphill *et al.*, 1942; Cleghorn *et al.*, 1948; Mikkelson and Hutchins, 1948; Altschule *et al.*, 1949; Parsons *et al.*, 1949), it seemed that ACTH might alleviate the picture. The effect of 4-hourly injections of ACTH in 8 patients with severe depression over a period of 7 to 17 days was not encouraging; there was no sustained beneficial effect, and E.C.T. had to be resorted to, to effect improvement

not explain. In 1947, when 38, she suffered a compression fracture of L.1, being thrown from a tram. She was in a cast a hundred days, became tired and irritable and lost her zest for needlepoint. The B.P. then was 134/80. Hypothyroidism and leukoderma were diagnosed.

In 1950, freckle spots appeared on her face. She got adrenal cortical extract (A.C.E.), but without improvement, became nauseated and weak, and was hospitalized in October, 1950. B.P. 88/68, B.M.R. — 14 per cent, corticoids 29, ketosteroids 0.85. Discharged on NaCl g iii and methyltestosterone.

In February, 1951, she returned to the Royal Victoria Hospital drowsy, weak and nauseated. The water test was positive, glucose curve flat. Serum sodium 277 mg. per cent. and potassium 25 mg. per cent. The B.P. was 66/36 and the ACTH test negative. She was given saline and A.C.E., and then cortisone 125 mg as an initial dose, and 25 mg. a day for 11 days. It had a dramatic effect. Her voice became stronger, and she appeared more alert, vigorous and euphoric Her extremities warmed up, and she developed a strong transference to her interne. It was found that she was hallucinating at night after her midnight dose of cortisone when her extremities and her mental processes warmed up. It was like a drink on an empty stomach. Her sex desire became strongly awakened, and she fantasied in this field, but was also disturbed by it. Rorschach done after 5 days on cortisone was severely pathological. Her physical state declined when cortisone was reduced after 10 days to 12.5 mg. daily, but hallucinations persisted and the idea that she could affect others appeared; also a paranoid element. About this time, she spoke of being sexually aroused at a party at the New Year, a unique experience in 10 years. After 6 days she was put back on 25 mg. cortisone daily for 6 days, as 12.5 mg dose was inadequate. She became more tense under this regime, felt trapped, and possibly suicidal Ideas of sex were prominent. After 6 days, cortisone was cut to 12.5 mg., and next day, DCA 2.5 mg. added. This was followed within 24 hours by the appearance of depression, and subsidence of sex feelings, and less fluctuation of mood. She also was secretive about fantasies, and

cortisone. This case which has been cited in my previous paper had the following history: pregnancy at 23 with pre-eclampsia followed by a three-month period of a salt-free diet and breast feeding her child. Six years later she became pigmented and amenorrhœic and soon after nauseated and weak. Addison's disease was diagnosed. On NaCl and DCA therapy she carried on, but developed a paranoid state with hallucinations in the summer of 1949. There was the usual E.E.G. abnormality. Her mental status did not improve with fresh pellet implantation, but did apparently with lipoadrenal extract, but relapsed. Finally, she was placed on cortisone 50 mg. a day. This seemed to lead to an exacerbation of her abnormal mental state. She became agitated and more deluded, and after 6 days, depressed. With an increase in the level of cortisone to 100 mg. daily, improvement occurred, and she became rational and cheerful, though the E.E.G. remained much the same, except it became insensitive to hyper-ventilation.

A case which illustrates some interesting features is one I saw at Dr. Browne's request, and the information I have is garnered from the ward record and other doctors' observations as well as my own. It indicates that psychotic features may apparently be brought out in an adrenally insufficient woman given cortisone to restore her cortical steroid level to normal. This patient claims to have had the menopause between 27 and 29 years. She then lost all sexual feeling. She was, however, active, did a lot of walking, reading and housework, and was compatible with her rather passive husband whom she had married at 27. In 1941, at the age of 32, she broke both legs while skiing. That summer, though she gained 14 lbs. at camp, she noticed an abnormal tan developing on her hands and a spot on her face. The latter disappeared in the winter. In 1944, when 35, she says the Addison's disease really started; she suffered bouts of diarrhœa and vomiting every 3 months, and would pass ¼ in. white worms. Investigated in February, 1945, she was found to have no free HCl in her stomach, and the B.M.R. was minus 19 per cent. The hæmoglobin was 72 per cent and eosinophils 15 per cent. After that she would have slight nausea and dizziness on the street which doctors could

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felt very somnolent and slept an unusual amount. Depression continued; negativism became apparent, though she responded with pleasure to a visit from her brother. Hallucinations of men in her room at night continued. The DCA was discontinued and the cortisone regulated to a dose twice weekly. She was enthusiastic about going home.

Summary

The evidence from syndromes of known hyperfunction of the adrenal cortex and from certain others in which it is implied, indicates that mental disturbances of varying degrees occur. These may even be so severe as to be termed paranoid schizophrenia. The associated cosmetic and other alterations in the body image are doubtless a psychic trauma and may be a cause of the psychological deviations. The fact that operation has been followed by improvement in the mental status before physical rehabilitation, and that in certain cases, mental symptoms have preceded the physical is considered evidence in favour of direct hormonal action on the brain, as the major causative factor. Mood changes accompanying the exhibition of ACTH and cortisone occur in diseases both with and without serious physical disabilities. There seems to be no consistent change, as both mania and depression are seen. Though psychotic states produced by these agents are generally transitory, they may be very persistent. A case of a catatonic-like state is described which did not show a remission following cessation of treatment, and a precarious one with E.C.T. The production of psychotic like states has been suggested as occurring in predisposed unstable individuals. More evidence in this area is necessary, as at least one contradictory case exists in the literature. Rectification of the psychotic state in a patient with hypopotassæmia has been referred to. The use of ACTH and cortisone has not met with much success in psychiatric cases. The treatment of adrenal insufficiency in the cases cited, led to an increase in mental symptoms in two and the appearance of fresh mental abnormalities in the other. More diligent psychiatric appraisal of all patients receiving ACTH and cortisone is necessary in order to increase our knowledge.

EFFECTS OF ACTH AND CORTISONE ON BEHAVIOUR

J. S. L. BROWNE

First of all I want to apologize. I know practically nothing about this subject, and I am not a psychiatrist. In terms of what Dr. Reiss, Dr. Pincus and I consider to be one of the possible things to do, I would like to tell you of our studies on the effects of these substances in a few cases. There are, I still think, great difficulties in terms of our re-orientation about the very rapid changes in mood which can occur under ACTH and cortisone. Psychological tests like the Rorschach are [illegible] that person does [illegible] you do get thes[illegible] st, then the problem of repetition of those tests at short intervals and the validity of those repetitions seems to me to come into question. Again may I emphasize that I know nothing about them, and therefore I do not know how frequently a Rorschach can be repeated with justifiable results. The fact of the matter is that we have studied a certain number of cases, some of them endocrine, others non-specific diseases, and have had repeated so-called objective tests done, and out of all this there is only an artistic impression, which I will show you, and which I think is much better than anything that I could describe to you.

Considering the effect on various steroids of the injection of ACTH, the corticoids, determined by the formaldehydogenic technique, rise, and so do ketosteroids; and with the cessation of ACTH there is a counterswing—the [illegible]

tomatic behaviour in the sense that he may become "driven" —this is the best word I can use—by the ACTH or the

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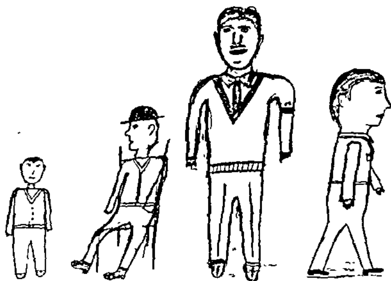
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day that he burst out against tl
of aggressiveness as compared
this is the most revealing thing



FIGS 1–4.

Male figures drawn by patient with beryllosis, treated with pork ACTH, 100 mg./day.

FIG. 1.
FIG 2.
FIG 3.
FIG 4

was drawn two months after ACTH was stopped. He was outside the hospital and this time he introduces walking for the first time. T

Fig 5 is the ter ten days ACTH. ure after 21 days. Observe her voluptuousness, the lipstick and so forth. I consider this is a far better representation than anything I could talk about. Fig. 8 was drawn two months after cessation of therapy. The size has regressed, and she has become slightly less feminine. I leave to you who understand psychology far better than I do, the intimate

cortisone, and then "undriven," depressed, or weak, almost Addisonian-like, for a week or two afterwards, depending upon the time which it takes his pituitary to recover.

A man with beryllosis was given ACTH and was greatly improved: his maximum breathing capacity rose, he could go upstairs, which he could not previously do. He had been an industrial casualty of a certain company in the United States, and he was a very meek and mild individual, who did not manifest any aggressive or hostile tendencies towards the company for the accident. He had been looked after by the company's doctor, and was pensioned. On the 21st day of treatment, the company doctor came to see him, and just as the doctor entered the room the man burst out in terms of the most intense aggression against the company, against the doctor, against all those things which he had apparently deeply resented. The Rorschach and other tests showed concealed hostility before treatment and this came out forcibly under the treatment.

That is my impression of the effects of ACTH and cortisone, that they bring out more intensively those things which are already there. I know that Dr. Cleghorn does not agree with me, that he feels that new things can be added. I can retreat into the point of view that in the child all the aspects of psychosis are present in an unformed manner, as is revealed by fingerpainting; and if you compare the fingerpaintings with Prinzhorn's book on "The Art of the Insane," you will see that in the latter the crystallization or hardening of the same artistic concepts are apparent in the artistic reproduction of these patients.

The psychologists (under Dr. R. Malmo of the Allen Memorial Institute, Montreal) asked this man at the various stages in his treatment to draw a male and a female figure. Fig. 1 depicts the male figure drawn before ACTH. Remember that this is not a patient with endocrine abnormality, except in the sense that he had a chronic disease. The man drew this himself; it is spontaneous; I did not even touch them up, nor have I altered the size. Particularly I would like you to see how empty this figure is in terms of its connotation. After 10 days of ACTH, observe the size increase and the introduction of motion (Fig. 2). Fig. 3 is the figure drawn on the

away again, and were restored by an implant of testosterone pellets.

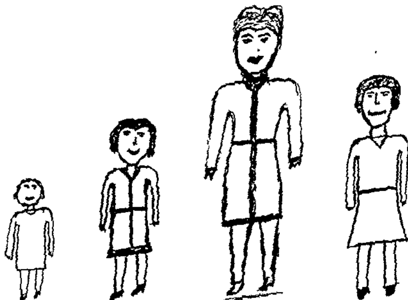
Then he became very ill: he had a sacroiliac osteomyelitis. We tried to feed him with a Miller-Abbot tube to try and reduce his vomiting, and we gave the milk-shake into the wrong part of the tube, with the result that he had an intestinal obstruction and he had to be operated on. After all this fuss and bother his pituitary was reactivated for one whole year, his basal was normal without any treatment, his sexual drive was perfectly normal, and his beard, which had not grown under testosterone at all, reappeared. Then he declined again, and returned to his former state.

H [illegible]
say [illegible]
him [illegible]
and I thought he was getting hypomanic. He was. And he became hypomanic on the tenth day of each treatment of ACTH that we gave him We are now examining him more carefully, giving him first pork ACTH, then beef, and then sheep ACTH, for experimental reasons. His drive on the pork ACTH was greater than his drive on beef ACTH. In terms of animal assay the effect of the two types of ACTH on urinary corticoids was the same, but he behaved differently. I think I can best illustrate his hypomanic tendency by telling this story. He had no money. He came in in a coma, and we always had to give him adrenal cortical hormone extract. That cost a great deal of money. We were paying for it, but the hospital always presented him with a bill, which depressed him very deeply. This time when he was under the ACTH the bill was presented to him. He looked at it and said "Say, you guys must have been painting my arse with gold when I was asleep!"

It seems to me that one has to ask patients to describe their feelings not only in the form of drawings, but also in their own words, and I think you have to get them to do it both on and off the treatment, because their views will be quite different in the two situations I haven't done that, but I think if one is going to explore this problem properly one has to ask people to do it. He came in in coma. Then he says (this letter was written under ACTH): "I was put on a bland

interpretation of these things, but I think they would form study in themselves of many years.

Next we come to a case of panhypopituitarism, whom have known for many years. He had an organic lesion of hi pituitary, a chromophobe adenoma, and he had testicula atrophy, and absence of libido and of adequate intercourse He had a basal of −40 per cent, and yet he was a *telephon*



FIGS. 5–8.

Female figures drawn by patient with beryllosis, treated with pork ACTH, 100 mg./day.

FIG. 5. Drawing before treatment.
FIG. 6. On 10th day of treatment with pork ACTH.
FIG. 7. On 21st day of treatment with *pork ACTH.*
FIG. 8. Drawing two *months after cessation* of treatment.

linesman in the province of Quebec in Canada, *where it* gets cold in the winter, and did perfectly well. *How h*[illegible] I *do not know*. He [illegible] *as one might expect* [illegible] hospital with a hypo[illegible] when he had an infection. Twenty-five milligrams of testosterone every two days restored his libido *and* his sexual *drive*. *On* cessation they went

One wonders whether the inadequate Rorschach was the reason why I felt that he was so much less intelligent than the Professor, because he did not react to his environment as much.

The patient who was a Professor of Philosophy was a panhypopituitary case, with a chromophobe adenoma. He had been treated with testosterone and thyroid for many years, had been improved somewhat by this, but again the difference between the effect of testosterone and the effects of ACTH and cortisone are most striking to the panhypopituitary patient. They both said that they become alive for the first time under ACTH. At this stage he was extremely anxious; he was driven. Things were quite abnormal to him, he could not sleep. He became conscious of anxiety. The Rorschach shows that he had this before, but he became more aware of it.

In a drawing under cortisone, which is what he felt best on, he increased the amount of head hair and also introduced a beard into the figure for the first time, as dots on the side of the face. He did not himself grow a beard. There was a difference in the degree of movement in the figure, in the aggressiveness or outward contact. One had the sense in him (not that he was a schizophrenic at all) that there [illegible]

He would not read books; philosophy books beside his bed were closed. Under ACTH he read about three philosophy books per day. He felt completely changed, and I would like to read you what he himself felt. He developed a number of anxiety symptoms, spells of numbness, paræsthesias, and so forth, under this, but he says, "The common felt effect of both (that is both ACTH and cortisone) has been a renewed energy and general feeling of greater liveliness. This has been most noticeable in the morning, before and after breakfast. Also observable was a less need of rest and sleep, especially during the day. A feeling of activity or capability of activity in the evening, which has always been one of my characteristics, was enhanced. Among the different felt effects of the two drugs were the following: this evening tendency to

diet" (we put him on a control diet) "after which I got the ACTH diet" (which was our control diet for metabolic reasons) "and was told that I had to retain my meals for six days before starting the ACTH" (he had a tendency to vomit). "I finally reached the sixth day without vomiting and the ACTH was begun. I didn't feel any different for about five days" (his response, as far as urinary corticoids were concerned, was slow), "although people could notice the *difference, inasmuch as I was talking more quickly, louder,* and could find the words which I wanted to say. From the fifth day on I felt much stronger and was full of vigour, until about the tenth day I felt Goliath had nothing on me. During the seven days intermission" (there was a pause in treatment) "I felt wonderful, but I think I toned down just a little" (the corticoids remained rather high for about five days, and then declined, which is unusual, as they generally fall within 24–36 hours of cessation of ACTH, and his psychological reactions corresponded to that). "I am writing this as I am nearing the end of the second series" (this was beef ACTH) "*of ten days. I have one more injection at midnight, and I* am right back shaking hands with Goliath." In terms of the objective tests he was an extremely interesting person. I had not regarded him as intelligent.

The next case I am going to quote you was a Professor of Philosophy,

than the pre

fessor's was

the fact that both these people were panhypopituitary people. Their rate of speech and their whole reaction were completely transformed, and they felt themselves to be completely trans-

the factor of time in responding. The linesman's Rorschach, however, *was extremely inadequate; he only* had about nine responses. This did not alter greatly under the ACTH, in spite of the impression that he became hypomanic. It is true that colour response was introduced under the ACTH—it hadn't been there *at all before—but I am incompetent to* discuss this technically, and so I had better leave it alone.

CLEGHORN: I think it depends upon the individual patient. For example, the first panhypopituitary that Dr. Browne described, is a man who I would say would be very unlikely to be doodling at any time, and this was a drawing which was required of him.

ZUCKERMAN: I take it that psychiatrists have tried to make a group of people like us do drawings on, let us say, every Monday at 10 o'clock for a period of three months?

BROWNE: I rather doubt that controlled experiments of this sort, asking normal people to do this performance at regular intervals, have done. I may be quite wrong. I just don't know.

LEWIS: I am pretty sure they haven't been done There is a lot of work available on children and their drawings, but I am not sure that the intervals, period of development and other variables have been controlled adequately. Analysis is very meagre indeed.

As to whether there would be opportunities for learning in between, and profiting by practice or instruction: I suppose that at the Allen Memorial Institute, where they take a keen interest in finger painting

normally.

BROWNE: I might add that these people were not in the Allen Memorial Institute; they were in the medical wards.

ZUCKERMAN Professor Cleghorn, in what proportion of cases which you handled with ACTH and cortisone, did these mental changes occur?

CLEGHORN. I should state quite clearly that we have not treated a large number of cases with ACTH or cortisone at the Allen Memorial Institute. We treated a series of eight cases of depression as an experiment to see if there was any alleviation. We have treated an

receiving large amounts of cortisone or ACTH over a period of time, to which we have not felt justified in subjecting our patients so far.

BROWNE We have been treating about 400 cases with ACTH or cortisone in all sorts of conditions, and about 125 have been adequately observed for this purpose, and of those seven became psychotic I would include Dr Cleghorn's case of dermatomyositis, who became withdrawn; the other case he mentioned, who became more paranoid

greater mental activity became, a few days after the treatment with ACTH began, somewhat excessive, the thoughts and ideas suggested by reading kept me from mental relaxation at bedtime and prevented sleep on one or two occasions, in spite of voluntary efforts to escape them. I have not experienced the latter effect as a result of the cortisone treatment. I felt ready to relax and sleep readily at 10.30 p.m. or 11. After three or four days of ACTH injection I experienced a dull numbness in the head, especially in the evenings. This felt like a superficial numbness, and did not interfere with thought processes or reading, at first. There has been none of this since the cortisone." Then he says "On the whole, then, the cortisone made me feel much better and has not left me with sensations and feelings which are foreign to my general make-up. Some of my experiences after ACTH were unusual and somewhat abnormal, for me at least." Again the Rorschach tests showed deep anxiety and showed preoccupation with somatic concerns. They did not change particularly.

This again is true of another patient, who went into a deep depression the day after the psychological tests were done. They had been done some months before, and then were done again, under the ACTH, and the amount of change in them was minimal, and yet that patient went overtly into a deep depression the next day.

So that even with the psychological tests we have used it seems to me we can't always characterize these patients adequately. They don't appear to change from their original make-up, but things are brought out.

DISCUSSION

ZUCKERMAN: What are these drawing tests, Professor Browne?

[illegible]
by [illegible]
Rc [illegible]

CLEGHORN: The "draw a man test," because that is essentially what it is, is done with a minimum of instruction.

[illegible] in between times? Had they got [illegible]

Supposing I treated a patient as successfully as apparently these panhypopituitary patients were treated, and he recovered from his mental symptoms, and then I had to stop treating him because we hadn't enough ACTH or cortisone. Is it legitimate in these circumstances to go ahead in the first place? It is a serious problem First of all, you are aware you may aggravate the mental state of the patient by giving him the drugs, and later on you may have to tell the patient that you can't enable him to remain well because you haven't enough of the remedy.

BROWNE That is one of my horrors: the patient is washed up on the beach with one wave and washed off into the sea again. This is one of the emotional problems which arise with ACTH and cortisone I fully agree with Dr Lewis that this is a very serious problem, but I don't know what else you can do Take for example the case of leukæmias in children—an encouraging newspaper article created much unhappiness and misery One has to have a consultation with the family doctor, who can point out that the disease is not one that can be cured, and that in time the child is going to fall back into the sea. I don't see that there is anything else to be done

ZUCKERMAN. Your answer then to Professor Lewis's question is that the decision ultimately is passed to the patient or the patient's relatives?

BROWNE· As it always is, in an operation, or anything that you try to do. This is no different.

ZUCKERMAN· But Professor Lewis has indicated that some of the reactions which you have been discussing are non-specific in the sense that other substances might also prevent the changes.

BROWNE Oh quite.

ZUCKERMAN: If that is the case, why then are you justified, may I ask, in using ACTH?

BROWNE· Well, because it happens to be a mysterious substance—
[illegible]

in that its effects spread out over a vast area of medicine.

ZUCKERMAN. Unique in the sense that here we have an agent which influences an enormous number of bodily processes, in brief, the entire metabolism of the body. But surely Professor Browne, there are other aspects to the general view you are stating. Metabolic processes can be interfered with wholesale by other methods.

BROWNE· This is just, to my mind, the quality of the adaptation syndrome, of what the body does in response to stress I would agree entirely with Dr Reiss that other glands are involved, but there is here a breadth of effect metabolically that I personally have not previously seen You will remember that at the International Physiological Congress in 1938 at Zurich, the primary effect of the adrenal was either on sodium metabolism, water metabolism, carbohydrate metabolism, etc., but now surely all these are secondary. This, in other words, is just like the blind man and the elephant

20 had no change at all in their mood; and about three have become very depressed and withdrawn. So one gets what perhaps is the normal distribution of psychosis in the population.

ZUCKERMAN: This euphoria is more or less specific, in your view, to ACTH treatment. You mentioned the fact that the Jesuit professor had already suffered from a number of therapies and that these did not produce the euphoria which ACTH did.

BROWNE. Your semantics are excellent. He has suffered and yet he hasn't suffered, because he was able, under testosterone and thyroid, to write an entirely new series of lectures on Kant, for example, but with great difficulty.

ZUCKERMAN. You, Dr. Browne, indicate that this treatment brings out what is already there—it intensifies an existing pattern of activity—as opposed to Dr. Cleghorn's view that new mental components might be created.

LEWIS. I wonder whether the difference of opinion about the extent to which these are new phenomena or old phenomena made manifest, is a real one. I can't quite see how it could be. You can't add anything to people's mental content and functional capacity, you can only reduce them or arrange new patterns of manifestation, and I suppose that the behaviour these patients exhibited might be evoked under other circumstances by many other influences, and must therefore be regarded

those phenomena were specific to the ACTH or the cortisone effect.

power or efficiency.

There is a further point I would like to ask about, and that is one that bothers us here, although it is perhaps not so troublesome in the U.S. and Canada It concerns the paucity of available material.

PART IV

EFFECTS OF HORMONES ON THE NERVOUS SYSTEM

MECHANISMS OF HORMONAL ACTION UPON BEHAVIOUR

FRANK A. BEACH

ONE of the first generalizations to be made concerning the effects of hormones upon behaviour, is that these effects are almost certainly numerous and diverse. It is unlikely that any type of behaviour is affected solely by one hormone, and it is equally improbable that a given hormone has one and only one effect upon behaviour. In addition, the influence of a hormone upon a particular behaviour pattern often is mediated by several independent intervening mechanisms.

It has long been fashionable to refer the behaviour effects of hormones to the nervous system and to suppose that when an endocrine product produces changes in behaviour, it does so by altering the functional activities of neural factors controlling the behavioural response. I believe that this is often an accurate assumption, but it is exceedingly difficult to prove because of inadequate understanding of the

to review a few studies that indicate ways in which behaviour is altered through changes in extra-neural structures.

Extra-Neural Effects of Gonadal Hormones

One experiment (Beach and Holz, 1946), involved measuring the behavioural responses shown by male rats which had been castrated at different ages and then subjected to

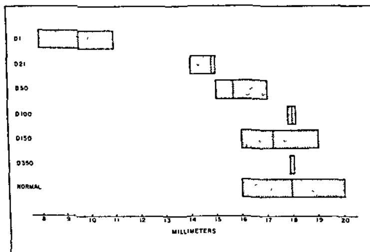
BROWNE: Yes. The adrenal, however, you have always with you.

PINCUS: I think that from a purely methodological point of view, the criticism which I have of all these observations is that they are not controlled. You say that you have the adrenal always with you, but in the case of the Addisonian individual or the adrenalectomized individual you don't have the adrenal, and you could give those individuals the placebos and see whether you get the same remarkable psychological changes that you get with cortisone. I am not aware unfortunately of any such observations. We have attempted that in Worcester with arthritic patients, and to our great interest, but not surprise, placebos have been extremely effective in producing euphoria, relief from pain, which to the psychologists looked very real. And so until one sees—and I think this is what Professor Zuckerman was leading up to—controlled observations on the actual medication that you are using, you are, Dr. Browne, being a mystic.

ZUCKERMAN: Dr. Browne, I have been permitted by Dr. Wolstenholme to select a topic for debate to-morrow, and it is obvious from my interest that something not dissimilar from this discussion is at the back of my mind. All I wish to say further now is that I have noted some of the language that has been used in describing certain clinical states, and I wish to refer to this matter here because of the contradictory nature of the impressions I have got.

The language used today to describe the mental state in Addison's

We believed that the infantile size of the penis in our Day 1 males, was responsible for their failure to achieve intromission and, therefore, for their inability to ejaculate. To verify this assumption, we observed several intact males in a series of mating tests and then removed several millimeters of the penile bone. Post-operative sex tests revealed that non-castrated rats with transection of the os penis behaved in precisely the same manner as had the Day 1



[illegible]

castrates. They showed a high frequency of incomplete copulatory attempts, achieved intromission rarely, if at all, and never displayed the ejaculatory pattern.

Here then is one obvious though indirect way in which testicular secretions can affect behaviour. In the rat, at any [illegible]

A second type of hormonal effect upon behaviour is revealed in the results of a study of changes in the glans penis in

androgen treatment three months later. Various groups of males were castrated at the following ages: 1, 21, 50, 100, 150 and 350 days after birth. After a 3-month interval, all animals were tested for their responses to a sexually receptive female, and then treatment with testosterone propionate was begun. In the absence of testicular secretions, the behaviour of all groups was approximately the same. Some excitement was aroused by the œstrous female and occasional attempts to mate were observed; but, in general, reactivity to sexual stimulation was quite low.

Under the influence of exogenous androgen, all castrates evinced increased excitability and, with the specific exception of rats castrated on the day of birth, daily injections of hormone eventually stimulated complete copulatory behaviour. Rats that had been castrated at ages ranging from 21 to 350 days, displayed normal frequencies of the copulatory and ejaculatory responses. Behaviour shown by the males operated at the time of birth (Day 1 group), clearly revealed a strong increase in erotic excitability. As injections continued, these animals made more and more numerous attempts to mate with the test female. However, the execution of complete copulation (which includes intromission), was quite rare, and the ejaculatory reflex occurred only once in a single individual.

Seeking an explanation of this peculiar behaviour, we found that in Day 1 castrates, the shaft of the penis was greatly foreshortened. Fig. 1 shows the relative length of this part of the copulatory organ in several animals from each experimental group. Males castrated on Day 1, plainly constitute a unique case. Two additional facts should be noted. Penis measurements made just before hormone treatment began revealed no striking differences between groups. Measurements taken at the conclusion of the experiment showed no difference between groups in the size of the glans. The suggestion occurs, therefore, that small amounts of testis hormone secreted during the first three weeks of life, prepare or condition the penile shaft for further growth after puberty. And if the initial conditioning does not take place, then the additional growth will not occur when androgen is administered later in life.



Fig. 2 Integument of the glans penis [illegible] normal male rat

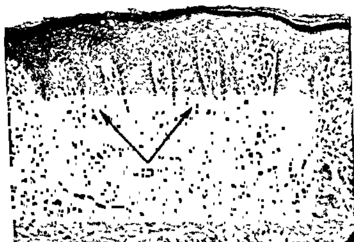


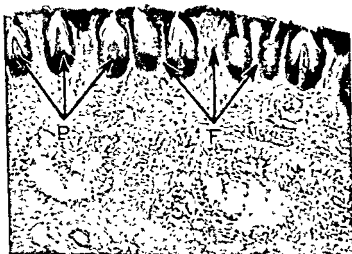
Fig. 3 Integument of the glans penis in a male rat castrated for 28 days and given no hormone treatment. "P" indicates remnants of former genital papillæ H and E ×230 (Beach and Levinson, 1950)

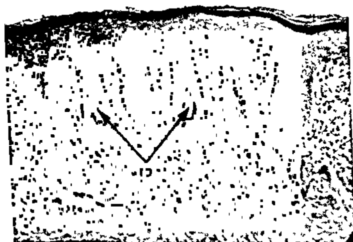
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castrated rats (Beach and Levinson, 1950). *Fig.* 2 illustrates the structure of the integument of the glans in the normal male, and Fig. 3 shows the changes that take place as a result of castration. The important features in these figures are the soft folds of epithelial tissue alternating with hard, cornified spikes or papillæ. Gonadectomy results in the total disappearance of the latter structures and renders the circumference of the glans smooth. Rats that are injected daily with 75 μg. of testosterone propionate show no deterioration of the integument of the glans. A daily dose of 25 μg. is nearly as effective. Five μg. per day retards, but does not prevent the gradual disappearance of the cornified papillæ.

Figs. 4 and 5 reveal the presence of touch corpuscles subadjacent to the papillæ. Discovery of these nerve endings suggested the following hypothesis. When the rat penis becomes tumescent, its periphery must expand. Under such circumstances, the soft folds of epithelial tissue will be stretched laterally, and their height will, therefore, be decreased. The cornified spikes, being more rigid, will not be thus distorted, but will now project above the tops of the adjacent folds. During coitus, the projecting spikes, rubbing against the vaginal walls, will be displaced, first in one direction and then in another. As a result of this movement of the spikes, the tissues lying directly beneath them will be distorted or compressed and this change, in turn, will give rise to discharge of nervous impulses from the touch corpuscles.

It is suggested here, that in the absence of testicular hormone, the penis loses one important type of accessory sense structure, and, therefore, becomes less sensitive to tactile stimulation. If this were the case, it would be legitimate to expect that partial loss of the genital papillæ might be accompanied by partial reduction in sexual behaviour. Fig. 6 reveals that this is indeed the case. The proportion of a group of castrated males continuing to copulate post-operatively, is here seen to be a function of the amount of androgen supplied by daily injection. And at the same time, the relative frequency of genital papillæ is quite similarly affected by comparable hormone treatment.


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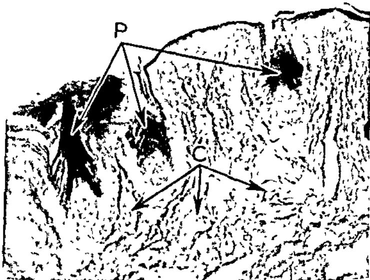


FIG 4 *Integument of the glans penis in a normal male rat.* "P" indicates genital papillæ "C" indicates tactile corpuscles. Bodian stain, ×375. (Beach and Levinson, 1950)



Effects of Hormones on the Nervous System

There is very little direct evidence to prove that hormones act upon the nervous system, but there is some reason to believe that this may be true. One of the most recent reports is that of Weiss and Rossetti (1951). These investigators studied changes in Mauthner's cells, which lie in the larval hind-brain of the tadpole, and are concerned with swimming responses. Under normal conditions, Mauthner's

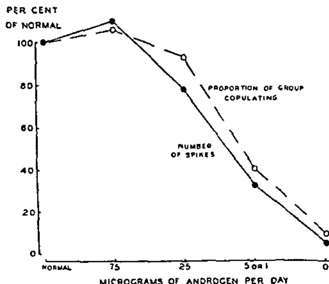


FIG 6 Effects of castration and androgen treatment upon the

cells disappear after metamorphosis, although other neurones which surround them continue to grow. Weiss and Rossetti implanted fragments of rat thyroid or bits of agar soaked in thyroxine into the hind-brain of immature tadpoles. Within a few days, the Mauthner's cells began to atrophy, while the surrounding

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on different types of neurones. Since the cell groups studied appear to have different functions as far as behaviour is concerned, it is permissible to interpret the findings as bearing directly upon the hormonal control of organismal reactions.

It is conceivable that pre-natally secreted gonadal hormones might act as "organizers," influencing the laying down of nervous connections which later are involved in the mediation of sexual behaviour. However, I have found (Beach, 1945), that normal mating responses can be evoked in female rats despite congenital absence of ovarian tissue. All that is necessary is to inject such animals with appropriate amounts of ovarian hormone.

Various writers have suggested that hormones may stimulate specific neural tracts or centres responsible for particular types of behaviour. Bard (1939), for example, is inclined to believe that œstrual behaviour in the female cat is evoked by action of œstrogen upon essential hypothalamic centres. And Kent and Liberman (1947), believe that progesterone may exert at least part of its behavioural effects in the female hamster by producing local changes in the brain. The major obstacle to satisfactory tests of such theories is our general ignorance as to the nervous basis for the behaviour under consideration. Until we know more concerning the location and identity of the neural circuits responsible for sexual behaviour, it will be very difficult to analyse the possible control of their reactivity by hormonal substances.

At present it seems reasonable to suppose that gonadal hormones tend to produce an increase in responsiveness to sexual stimuli. The most carefully and extensively studied species are the rat and guinea pig. In the former, the effects of ovarian and testicular hormones are certainly not confined to the cerebral cortex, and, as suggested in the first paper in this volume, there is reason to believe that they take effect at subcortical levels. Much more extensive investigation of the neural basis for reproductive behaviour will have to be made before any more specific or useful interpretation of hormonal action on the nervous system is likely to be advanced.

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DISCUSSION

PINCUS. As far as copulatory behaviour is concerned, I don't feel competent to make a contribution. I think that Dr. Beach's data stand by themselves. I will, however, bring in two comments that have occurred to me on hearing him speak.

We have heard repeatedly the suggestion that perhaps the adrenal cortex may contribute hormonal factors, since it certainly is a steroidogenic gland, and under certain experimental conditions particularly, can be very highly productive of sex hormones. There have been mentioned at these meetings the very interesting experiments on the effect of very early castration, particularly of mice, in which with the development of adrenal tumours in certain strains one observes the appearance of sex cycles, particularly in the female, indicating a marked and adequate production of œstrogen by the adrenals, either by these tumours or by the adrenal tissue itself. So that consideration of other possible hormonal factors is certainly not ruled out and is one which requires further investigation.

The second point is the extent to which these hormones concerned with mating behaviour operate through the central nervous system. Now here we have the ver
rate, between the male and
is at least conditioned by
mating behaviour is not.
behaviour has its nervous component located somewhat below the cortex, whereas masculine mating behaviour is cortically controlled. At any rate, one of the questions which occurred to me, as a biochemist, is what is the mechanism whereby this hormonal influence upon nervous tissues is exerted? This is a question which one thinks of not only in relation to mating behaviour but in relation to all the aspects of behaviour that we have been studying here. Now there exists in the literature practically no information about this which can be called concrete. As far as I am concerned, the most interesting experimental data, giving a sort of lead to the situation, are those of

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observations because you, Dr. Beach, have also indicated a disbelief in the existence of specific nerve pathways that affect sex behaviour.

DEMPSEY: I would like to comment on the effect of lesions in the cortex of the two sexes. In the male, cortical lesions lead to loss of sexual behaviour, whereas in the female they do not. It seems conceivable to me that in the male, the excitatory pathways to sex behaviour go through a cortical mechanism. Certainly the animal's eyes and quite probably his nose, and possibly other sensory mechanisms are involved. One can comprehend, therefore, how decortication in the male might reduce the total amount of excitatory stimuli to the centre for sex behaviour. In the female, on the contrary, sex behaviour can be induced by a much simpler stimulus, namely a mere tactile stimulus to the back of the animal. Here decortication does not lead to [illegible]

sequent to this operation two female cats came on heat, mated, became pregnant and delivered kittens. However, they were unable to care properly for their litters. [illegible] one placed a kitten to her [illegible] the mother would lie down [illegible] licking movements with her [illegible] permit them to nurse for [illegible] contact there was no maternal behaviour at all. We interpreted this to mean that the only way the mother could recognize the situation was through this specific stimulus. I wonder if you will agree with this general notion?

BEACH: Yes, entirely. Confirmatory evidence is provided by experiments on peripheral desensitization.

Male rats are less likely to mate after removal of the eyes, cauterization of the olfactory bulbs, or transection of sensory nerves serving the snout and lips. But the same operations have relatively little effect upon the female's sexual behaviour. It appears that the cortex mediates integration of [illegible]

cortex.

FOLLEY: How do you measure maternal behaviour in rats? What are the responses?

Gordan and his collaborators, in which they demonstrated that castra-

quite large, much larger, for example than the doses of testosterone that Dr. Beach uses in ... wonders whether or not the effect studied by G... logical situation. *in vitro* conditions

testosterone) upon certain respiratory systems of the brain, one is left ... biochemical basis

make an approximation as to the mechanism whereby certain of these effects are exerted.

ZUCKERMAN: There may be point in referring to a collateral observation which indicates that some of these responses are far less specific than general. Dr. Beach will recall that many years ago Sherrington showed that spinal trans-section in a monkey will precipitate uterine bleeding. This observation was confirmed by Dr. van Wagenen in New Haven. I was first under the impression that in this response ... nervous pathways, and ... f experiments section of pelvic parasympathetics; dorsal roots, and so on. The more I experimented, the more general the response appeared, ... interference with the auto-

behaviour. John Fulton and I operated on a baboon, ... a small lesion in the hypothalamus. At the time of the operation the baboon had swollen sexual skin. As a result the sex skin collapsed.

The second site of origin for sexual disturbances is the hypothalamus, as is well known, and here again there are two groups: those in which there is an acute episode—e g , in a tumour of the third ventricle, you may get indecent attacks on people, just an episode with a moderate impairment of consciousness, and that's all—and the second group consists of those in whom the hypothalamic tumour produces virilism, with consequent disturbances of steroid metabolism and of behaviour.

At the lowest level, which is equally well known to all physiologists, are the cases of transection of the spinal cord, in which you get priapism in response to imitative factors in the pelvis It seems to me that observations such as these emphasize the importance of the neural pathways in all studies of sexual behaviour

Zuckerman: Many years ago, Dr. Le Marquand described a little boy who had a small neuroglial tumour, if I remember rightly, somewhere just in front of the pineal gland. It bore no relation to a tract of any kind, nor did it have any connection with the pineal. Nevertheless the boy manifested very aggressive sexual tendencies at the age of two or three. Would you regard this case, Dr. Elliott, as also indicating the operation of a specific mechanism?

Elliott: I don't know. We are collecting a mass of material, and we have yet to digest it, because only now that we've got the EEG to work with, can we know whether some of these episodes in such children are, in fact, epileptic or not.

Harris: With regard to the central pathways that were discussed just now, I was wondering whether the tumours in the paracentral lobules stimulated the sensory cortex and perhaps indirectly stimulated

might act as osmometers, and via the dendrites attached to them, might stimulate the cells of the supraoptic nucleus when the osmotic pressure of the blood is raised It is the only attempt I know to speculate on the mechanism by which a hormone affects the central nervous system.

Beach. In the first place, there is ample evidence to indicate that

to be found in human beings

BEACH. The females are put in a cage 3 ft. square, and 100 paper strips are hung from the top of the walls. Once a day we count the number of strips the rat has taken down and grade the excellence of the nest. When the young are born, we count the number that are completely cleaned of fœtal membranes, the number that are partly cleaned, and the number that are uncleaned. Devouring of placentæ is checked. Twenty-four hours after parturition we remove the mother and scatter the young about the floor of the cage. The mother is then returned and her retrieving behaviour is recorded. There are other tests, but these will give the general idea.

WALTON: I was very interested in this question of decortication of the male. In the analysis I attempted of the mating behaviour pattern of the bull, I think one could show quite clearly that the pattern was a consecutive process, in which, first of all, you have the mating behaviour of the male, consisting mainly of motor patterns, and when the motor patterr [illegible] that, ejacula [illegible] tor patterr [illegible] and so on, [illegible] the failure of the motor pattern, but does it necessarily follow that the processes of ejaculation and erection are also directly affected? I wonder, if on these [illegible] Moore's ejaculatory [illegible] autonomic system,

BEACH: It certainly is possible. In our tests an initial state of arousal must occur before these other things will appear. The cortical mechanisms seem particularly important for the arousal or erotic excitement.

REISS: I wonder if there is some relation of the phenomena to the [illegible] the blood content [illegible] with [illegible] m. [illegible] ilarly [illegible] iexo- [illegible] hole phosphorylation processes in the brain are also considerably increased.

Thirdly, we have during the last years collected considerable evidence that the brain stores considerable amounts of steroid hormones. To give you some idea of the quantity, the total brain lipid contains about 0·05 per cent of ketonic material.

ELLIOTT. It strikes me that there is a lot of relevant material in [illegible]

PART V

DEBATE ON METHODOLOGY

Zuckerman: As I indicated yesterday, it appears that some kind of gulf exists between the methods of the experimentalist (for example, the methods followed by Dr. Beach in the study which he recorded in his last paper) and the methods of the clinician, and there also appear to be differences between the methods followed by different clinicians. We have to accept the fact that in clinical practice ordinary controls are difficult. Consequently the clinicians use the method they call "internal control," that is the continuous study of single individuals rather than studies of groups. I have a feeling that Professor Lewis does not regard this limitation of clinical study as essential. There also appear to be differences in the way people treat individual cases. For example, Dr. Reiss, as far as I could make out, does not pay any particular attention to variations in the behaviour of the individual he studies; he accepts the diagnosis of the psychiatrist, and then subjects the patient to a battery of tests. On the other hand, in his discourse yesterday Professor Browne indicated quite clearly that he is interested in what he called an artistic picture of the behaviour of each individual as he varies in time.

I feel that the problem essentially boils down to this. We are accustomed to believe that there is only one method of science—the method of controlled observation. To make it work it is necessary to muster every bit of genius, every hunch, and all the artistry that can be brought to bear on the problem that is being tackled. The question is whether or not hunches alone are sufficient in clinical medicine, or whether there, too, we need the controlled observations usual in experimental science. Dr. Beach has indicated several times that in his view there is a serious lack of proper objective tests

ZUCKERMAN: Dr. Beach, when you were discussing the possibilities of some central action, you first referred to the possibility that the steroid hormones might [illegible]
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that the balance of evidence is against Carl Moore today. Burn's interpretation of Carl Moore's observations on the opossum is that his experiments were started after the reproductive organs had already [illegible]

injections should include a measure of the effects of injecting inert material. This sort of thing is realized by many clinical workers but it should be even more widely appreciated.

Cleghorn: In the investigation of such diseases as Addison's disease, where the clinical state and the life of the patient is critical, placebos must be used with great caution. When we have at our disposal a form of treatment which is life-saving and we are investigating a new drug, which is presumably life-saving too, we first have to establish the validity and the effectiveness of that form of treatment. There is a second point which is of importance, and that is the small number of [illegible]

of some sort as alternatives to cortisone, but one has to do that with a great deal of circumspection, because these individuals have a rather nasty habit of turning up their toes and dying with great rapidity. So that this introduces an element into this type of experimentation which makes for very considerable difficulties. I don't think the difficulty is perhaps so great in some other diseases in which life is not so critically in the balance. But we must first learn what these substances will do, and when we are satisfied that they [illegible] -ycho- [illegible] know [illegible] But this question of the welfare of the patient is a thing which we have to keep in mind. It is a matter which Sir Thomas Lewis has discussed some years ago very aptly, and is one which makes clinical investigation in many respects very difficult.

Harris: Mr. Chairman, I think you defined the subject for discussion as the difference in viewpoints between the clinician and the scientist, and I would like to start from there and from the observation that Dr. Beach made on Tuesday afternoon, that the data regarding the steriod hormones were of a different category from those regarding the behavioural reactions.

Firstly, with regard to the steroid side of the picture. The

of human behaviour. He wants to apply batteries of tests to men as he does to rats. Now one needs to be careful about the way different tests are correlated. We have been told that different psychiatrists usually agree closely in diagnosis. If that is the case, why then is it that the ordinary methods of scientific study cannot be followed in clinical investigation? I have asked Dr. Beach to open this discussion.

Beach: Experimentalists working with lower animals have many advantages over clinicians. For example, the experimentalist doesn't have to do preliminary research to discover the species of animal with which he is working. This has already been done for him by earlier workers in taxonomy. The first steps in studying animal behaviour consist essentially c [illegible] t [illegible] a pre-Linnæan job, so to speak. For many clinical syndromes we have not yet reached the stage of knowledge that will permit an adequate scheme of classification; more accurate description must come first. It may be that one important need is for a sharpening of definitions and of terms. Progress inevitably will be slow until our behaviour criteria are more definitive and our concepts more capable of objectification, so that one clinician can talk to another and mean the same thing, and clinicians can talk to non-clinicians and convey definite meaning.

I agree most heartily with Dr. Browne's emphasis on the importance of longitudinal studies. The current fashion in psychology to amass data on large groups of subjects and to deal with such data in terms of central tendencies and measures of dispersion is entirely worth while. But individual variability in terms of differences between people or differences in the same person from time to time forms an essential part of the total picture.

In contrast to experimentalists, the clinician is primarily concerned with the welfare of his patients, but this need not prevent application of some principles that have proven essential in experimental work. There is, for example, the matter of introducing simple control measures. As Dr. Pincus said yesterday, any study of reactions to hormone

published a rating scale, devised by Dr. Malamud and Dr. Hoagland, which appears to give objective rating of behaviouristic changes in schizophrenic patients and in other patients studied. This scale we have employed in studies on the effects of cortisone administration upon schizophrenic subjects, and also in what we call blind tests, in which neither the observer nor the patient nor the nurse knew who was getting what. The results on the whole have been extremely good from one point of view, and extremely discouraging from the other. We have followed longitudinally about eleven schizophrenics receiving a long course of cortisone. Only two patients showed by this rating scale alone any improvement whatsoever. All the others were rated day after day and showed absolutely no change as a result of the administration of this very potent steroid. But, regardless of the result to date, the point is that one can make an objective measurement, which one can express in mathematical terms, of certain types of psychiatric behaviour.

Concerning the endocrine complications which Dr. Harris remarked on, namely the fact that in any endocrine situation there is the possibility that one upsets a complex internal balance as soon as one administers any potent steroid or any potent pituitary hormone. there are available, to a laboratory at least, methods whereby the upset of this balance may be measured. [illegible]

nevertheless there are available laboratory methods which will allow us to decide, for example, whether cortisone is inhibiting the production of certain types of compounds in preference to others, or whether it exerts a general pituitary inhibition. Very recently in the States several investigators have put forward methods for the measurement of various steroids in the blood as well as in the urine. And all of these taken together offer, I think, from the point of view of the laboratory, at least a modicum of technique which will allow one to combine the endocrine attack with the clinical attack.

With regard to some of the remarks which Dr Beach has made, the use of various types of psychological tests in human

experimental results that have been given here have been obtained from work on animals that have usually been gonadectomized or adrenalectomized. A difficulty in the study of cortisone and such-like on the human is that one is usually dealing with patients with their own pituitary-adrenal axis *in situ* When you inject cortisone into these patients, firstly you don't know the effective dose, because you don't know how the injection of the exogenous material is affecting the patient's own secretion; and secondly, it is possible that the injected cortisone is blockading the secretion of other adrenal steroids, and that some of the effects observed are not due to a positive reaction on the part of cortisone but to an inhibition of other adrenal steroids.

Coming to the data on behaviour. In the laboratory, tests may be devised and various aspects of the total behaviour may be measured and given accurate figures. The value of that obviously is that the experiments can then be repeated under identical conditions, and put to the test for confirmation. In regard to human behaviour, in most cases the ability to measure it is lacking, and then it is necessary to give a description of the behaviour in words. This may be very difficult, especially when the terms involved are not well defined. For example, in the discussion yesterday on homosexual behaviour, it was apparent that the term "homosexual behaviour" could cover a variety of different behaviour patterns, ranging from that of normal children, of normal adults, of adults whose sexual activities are orientated exclusively to members of the same sex, and to pathological states such as that seen in cows with cystic ovaries. Here then is a single term covering a variety of different states. Also it seems likely that this term, taken as an example of many others, may have different connotations in different regions, and I should like to put in a plea that such words and phrases should be defined as clearly as possible in discussions, especially when workers from different nations are gathered together.

Pincus: I think that one can devise techniques which would meet Dr. Beach's needs. The need for objective observation is one which we have felt for a long time, and as far as observation of the psychiatric condition of the patients, we have

cases, it appears that under-functional, over-functional and normal states are equally distributed. The only thing we can say is that very often when very high thyroid function is found, the ketosteroid and cortin excretion is low, and mental improvement is accompanied by normalization of both these factors.

In schizophrenia, we found both hypo- and hyper-functional states of the adrenal cortex. And we are of the opinion that the great fluctuations in the functional state of the adrenals are very important in pathophysiological analysis. We see how these fluctuations stop when the patient is successfully treated with insulin or other treatments which are still in the experimental stage.

It appears highly unlikely to us that one will ever find identical biochemical or endocrinological disturbances responsible only for one special psychiatric disease entity, since we [illegible]

depends on the preliminary mental make-up of the patient.

Lewis: I think Dr. Pincus is quite right when he says that one can now observe objectively quite a lot of phenomena with a good correlation between observers. But I am not sure [illegible]
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to ar [illegible]
They can't be anchored to anything physiological. Such basis as we now have for objective assessment of personality lies in the [illegible]
ate [illegible]
eith [illegible]
and by experimental situations, control the change in behaviour and observe more satisfactorily for research purposes. But surely the chief difficulty in all this lies at the great variability of human behaviour at different times. This brings in the need for the internal control, as Professor Browne would say, for study over a long period.

Moreover, in psychiatric disorders you come up against a

experimental subjects can be practised, and is in fact practised in a number of places. The big difficulty there is the relevance of these tests to the particular aspect of behaviour that one wants to study. We have, for example, a collection over the years of an enormous number of Rorschach scores on schizophrenic patients, and I must say that the net result is that we do not know with what the variations in the scores are correlated. The psychologists tell us that this represents the colour sense, this is, shall we say, the measurement of aggression, and this is the measurement of this and that, but we as physiologists have as yet found none of our variables correlating significantly with their figures. There are other tests for which the measurement basis is firm and good, particularly the various so-called intelligence tests. Again, on an endocrine basis, there is no correlation whatsoever between any phenomena that we have examined and I.Q. What we are seeking, and what I think need to be devised, are tests in some other field. Perhaps one might call them "objective tests of personality." The only approach we have had to them so far, with the exception of one test which I can describe if necessary, has been this psychiatric rating scale.

Reiss: I should like to comment on Dr. Pincus's last remark, that there is no relation between I.Q. and endocrinological phenomena. A few years ago we investigated about 80 boys from a remand home and 80 boys from a public school, and found that the higher the I.Q. the lower the ketosteroid excretion, and *vice versa*. The higher the I.Q. the slower was the progress of sexual development. We found that particularly the boys in the remand home had a lower I.Q. and higher ketosteroid excretion [illegible] mean [illegible] more people became aware of this fact.

I have the same experience as Dr. Pincus, that one rarely finds an absolute co-ordination between a well-defined [illegible] te. Let me [illegible] ultaneously [illegible] essive cases. [illegible] of over 600

the same methods as for laboratory animals. The value of those experiments is questionable. I can tell you my feeling: no value at all, because if it is difficult to perform an experiment on an animal, it is much more difficult to perform an experiment of the same kind on man. Now the European point of view is that experimentation with an animal is just a means to solve a problem. I am not quite sure that, in shall we say, Bombay or Calcutta, people would quite agree with all that we have said here on experimental work on animals. You may smile, but there are millions of people who think the other way, that is to say, that even animals should not be used as a means to solve a problem. *We* think that men should not be used as a means to solve a problem, but there has been a social group which experimented on man for ten years. For what? Possibly for the welfare of the patient, but not for the individual patient, supposedly for the welfare of the people on the whole, not only for the people of today but for the people of the future. This is quite a new idea, and might disappear now, but maybe reappear later.

And as we have discussed homosexual behaviour, I would mention that during the Nazi régime homosexual behaviour had two sanctions: either castration or the camp. The theory behind castration was based on old laboratory work on animals, and here you touch upon a second problem, that of extrapolation from one species to another. It is known that a few thousand people were castrated on this wrong biological basis; others went to the camp, from where some came back. This shows that society may profit by what we are doing in the laboratories to put up new laws in a very wrong way. I think we face a paradox which is very difficult to solve. We know that it is not possible to extrapolate from one species to another, that is to say, even from animal to animal. Yet if you want to work on man as we are working in laboratories on animals, we shall have to change many of our ethical and social standards. I don't know where the limit is between experimentation and therapeutics—it is very difficult to say. I think the only man who knows is the man who performs the research work himself. He alone knows if it is for the welfare of the patient, and he knows if he is doing the work in order to clear up an interesting question or for a less allowable motive.

more practical difficulty—you can't deliberately give people a drug that will make them temporarily insane; it is indefensible. If you have unintentionally done so, and then succeeded [illegible]

with the method of internal control the method of observation of groups which has been carried out at Worcester. I would have thought statistical techniques appropriate and essential, when you have so many variables.

Klein: If you will allow me, Mr. Chairman, I should like to bring this question of methodology to another level, the level of social and perhaps moral implications. It appears that the main question you have put to us, is: "How does it happen that there are these big differences between the methods of the laboratory and the methods of human investigation?" Dr. Beach perhaps gave us a start in answering this. He told us that for clinicians the welfare of the patient is always the main preoccupation, whereas the laboratory investigator uses the animal as a means to solve a problem. I am glad to hear him saying that, but I feel that such a social way of thinking is that of a man from the United States speaking in London in 1951, it has not always been the same and will not be the same in the future Experimentation on man is officially prohibited in most countries of the world. I say officially, because what is going on behind the scenes is another question. But there has been one régime where experimentation on man was not only allowed but was even officially regulated And that was the Nazi régime. It is necessary to recall that. Some people would like to think that this never happened; but it has happened, and it lasted ten years. Endocrinological experiments were performed and on a very big scale. I should like to mention one name, very well known, Professor Clauberg. He is a man who had in former years been working on the endometrial test for the corpus luteum, and this man performed thousands of experiments on women, with quite definite tests and quite selected series in the Nazi concentration camps in Silesia (1944). It shows that sometimes experiments may be performed on man on the same scale and with

the same methods as for laboratory animals. The value of those experiments is questionable. I can tell you my feeling: no value at all, because if it is difficult to perform an experiment on an animal, it is much more difficult to perform an experiment of the same kind on man. Now the European point of view is that experimentation with an animal is just a means to solve a problem. I am not quite sure that, in shall we say, Bombay or Calcutta, people would quite agree with all that we have said here on experimental work on animals. You may smile, but there are millions of people who think the other way, that is to say, that even animals should not be used as a means to solve a problem. *We* think that men should not be used as a means to solve a problem, but there has been a social group which experimented on man for ten years. For what? Possibly for the welfare of the patient, but not for the individual patient, supposedly for the welfare of the people on the whole, not only for the people of today but for the people of the future. This is quite a new idea, and might disappear now, but maybe reappear later.

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I am just raising these questions. I don't know what the answers are. I am afraid that social evolution will impose on us an answer one way or another.

Zuckerman: Professor Klein reminds us very usefully that a general social climate of opinion also affects attitudes to science. He has indicated quite clearly that a lot of the work we do would be impossible in, let us say, India, where there is a completely different attitude to working with certain kinds of animal. But I imagine, Professor Klein, that in indicating that different social climates determine different attitudes to science, you would not attempt to qualify the view that science must always rely on controlled observation. One has to pay attention to the limitations which one's material imposes; but having paid attention, one's object, one's goal, remains the same. And here, Dr. Browne, I would like to throw the ball to you.

Browne: Mr. Chairman, I think the point lies largely in something you have just said. It is this: how far can one extend the number of variables which one can accurately observe? One can in physical chemistry maintain factors A, B, C, D, E and F constant, and then observe the behaviour of molecules, atoms and so forth, under these controlled conditions. In biology we have a tendency to say, let's assume that A, B, C, D, E and F are constant, and having considered them, then to ignore them, and then, within the assumedly constant environment, say that the thus and thus relations are true. That I think is necessary in biology because there are so many variables. But we are thereby constantly surprised because actually these factors are not constant, and they come round and hit us on the back, and subsequently our scientific colleagues discover that what we have neglected is, in point of fact, pertinent. That is then the inherent difficulty in doing experimentation on any species, and becomes even more difficult when one is dealing with man, because the internal environment of man—I am entirely unable to say, of course, whether the internal environment of the rat also includes fantasy and symbolic significance—but the internal environment of man does include the symbolic

significance of things which are done. And that is where Dr. Pincus's desire for the placebo or the control comes in. We have done a good deal of control injections of such things as cholesterol, saline, and so forth and the great difficulty is to make it feel exactly like ACTH. Patients can tell the difference, and so you do not have a real control. We have as yet to invent a substance which feels to the patient exactly like ACTH. So that makes it very difficult indeed.

One can then take the point of view that one must investigate what I would call in depth, that is to say, the constant refinement of a single test, the constant exploration for more accurate methods of doing it, the investigation of more and more steroid compounds, as Dr. Pincus has indicated is so desirable. One may say that one must in using an assay use 100 animals That is the desire of biologists very frequently. There is no statistical significance to a single assay done on, say, a 24-hour urine unless you use 100 animals. Well, you can't use a hundred animals on certain things because there is not that much active material in a 24-hour urine. Nor can you, in terms of doing longitudinal studies, use a hundred animals, because that becomes economically impossible for any one laboratory to do So you must constantly make a compromise between the intensification of study in depth and the study over a sufficiently long period of time to have the physiological function which you are investigating, for example, the menstrual cycle, repeated often enough for the individual variations in, say, pregnanediol output or occurrence of ovulation, to be seen. We have found from a year or two years' observation that a certain individual will ovulate twice, and we know what the chances are of them becoming pregnant. If in such a patient you give hormone X, and the whole thing comes together like chance, and the patient becomes pregnant, the pregnancy may be attributed to hormone X, whereas it may not have had anything at all to do with it. Consequently I think then, again it is a matter of attempting the very best possible control. I fully agree with Dr. Beach that control experiments are desirable, if they can be done without detriment to the patient. It is feasible, of course, particularly in the realm of gynæcological endocrinology, where the patient is ardently

desirous of having a child and one can therefore observe him or her, her principally, over a very prolonged period of time in which they're willing to co-operate. Where it can be done, it can be done very satisfactorily. But even the personal interrelation between the physician and the patient has to be considered in terms of its effects on physiology. It seems to me that we are again making a compromise between our consciousness of the number of variables which are involved and of the importance which they have for our particular study, and I think that what one should avoid is the implication that one can only be scientific by ignoring the variables which one cannot control. This is a most important point for us to

that giving a thing a number necessarily makes it more true. If in fact this number does reflect the rating of the observed quality, then that is all right, but I am terribly afraid of derived data, and I do wonder whether when we give things numbers, we do not thereby diminish our responsibility in considering their validity. I entirely agree that you can't go on keeping your mind suspended eternally between 1,000 variables, and must drop off hundreds of them and leave the ten that you can deal with at the moment.

Dr. Beach speaks of the pre-Linnæan school of descriptive validity. In medicine people have described things and have given them names, and in my opinion the giving of names is equivalent to giving data numbers. But if things do appear to fit together, we put them together and call them unique clinical entities, and now we have found that so many parts of them merge into each other. I find that ACTH and cortisone particularly have revealed this in medicine. As Dr. David Thomson, our Professor of Biochemistry, says, we used to think that truth was a convergent point which one could attain; now we think it is a black speck in the middle of a cloud, and are not quite sure that there is a speck there.

Dempsey: There was a point in the initial part of the discussion in which I would like to change the emphasis. It

seemed to me that the discussion took the point of view that there was a dichotomy between clinical investigation and laboratory investigation—that these two types of activity were qualitatively different. I would like to debate this point. Any experiment involves a situation designed to provide a logical answer to a limited question. There are practical limitations to any experimental situation. For example, it is difficult or impossible to perform certain surgical operations on mice because of their small size. The welfare of the patient imposes limitations on certain experimental procedures in human beings, whereas in other situations the human may be the ideal experimental animal. A great deal of our knowledge of neurology has come from the human because it is the ideal species for obtaining the particular kinds of information sought. I think I remember that Flexner once said there was no great difference between the scientific attitude and the clinical attitude, and that in both cases the intellectual operations were comparable. The scientist starts out with information derived from observation. After reflection upon this information, he performs an experiment which interferes in some way with the phenomenon. He then observes again to detect the results of his interference, and finally forms a conclusion. Similarly, in clinical medicine, the clinician observes a phenomenon, he reflects upon what he has observed, he interferes in some way by administering therapy, and again observes the results of his therapy. Finally, he forms a conclusion. Each individual phenomenon may be simple or complex; it may be difficult or it may be easy. The validity of the conclusion which is finally arrived at, however, is a matter of logic, not of method. The validity is determined by the rigour with which the intellectual process is carried out, rather than by the method itself. Consequently, I am not sure th [illegible]

doing [illegible]

labora [illegible]

means must be found to circumvent these difficulties. It seems to me that science, either in clinical or in laboratory investigation, boils down finally to the severest possible intellectual effort that can be made to understand a situation, rather than that it is resident in any particular method, or technique, or

apparatus, or procedure which is applied to the particular problem.

Walton: I think we haven't given enough attention to the possible use of statistical analysis in work involving observational data. Thirty or forty years ago statistical analysis was confined more or less to the determination of the significance of a mean, but with the very considerable advance of statistical methodology, we now have statistical tools which are becoming more and more adapted to the problems of multiple observation; and by analysis of variance and the use of *methods applicable to small samples; these tools are becoming* more and more available in the field of clinical observations. I think it is incumbent upon the clinician and all those who are engaged in this type of work, which must of necessity involve multiple observation, to see that the best use of that material is made by adequate statistical analysis.

Kalmus: Working on human genetics where statistical problems are more or less everyday problems, I would emphasize what Dr. Walton said, and would even go farther. I would say that statistical considerations must enter into any conclusive experiment before it is started. It is no good finishing one's experiments and then coming up to the statistician and asking what can be done with the results, because *then very often nothing can be done any more.* But if one plans beforehand after some initial groping, and chooses conditions and experimental layout carefully, the analysis of

world by scientists, but too seldom by clinicians.

On the question of a possible relationship between intelligence scores and endocrine state, there is of course a high correlation in respect of thyroid function (cretinism). However, I agree that in general one cannot hope to find high correlations between units or entities at different levels of organization. One cannot expect a one: one correlation between the intelligence and biochemistry of normal people. If, on the other *hand, one is dealing with a clear-cut defect,* something might eventuate on the chemical level. In this way

a small fraction of mentally defectives have been found to suffer from an inborn metabolic error, namely the inability to metabolize phenylalanine, which results in their excreting phenylketonuric acid and other abnormal substances. The difficulty in this field is to discriminate between the many etiologies of mental defect.

About 70 years ago Sir Francis Galton, the founder of the laboratory where I work, wrote his "Inquiries into Human Faculties." Anticipating quite a lot of psychoanalysis, he showed that human faculties, especially mental faculties, are really something worth studying in detail. The simple approach of correlating the stimulus at one end with some sort of total, preferably numerical, effect at the other end, so beloved by the behaviourist school, appears in this light rather childish. For us here the most relevant result of Galton's is that various actions of people, which appear to be identical, are nevertheless achieved by quite different psychological mechanisms. To give an example—mine, not Galton's—some people can play a piece of piano music from memory; others will play it from the printed sheet; and they may be guided variously more by the eye, by the ear, or by kinæsthetics. Any physiological analysis of piano playing which does not take account of these differences, is bound to go astray. In particular, if we want to study any influence of the endocrines on "higher" human performances, we must know more about the factors and mechanisms involved in these activities. Nevertheless, if I have learned anything from this conference it is the following: we shall probably get farther in psycho-physical studies if we start at the level of endocrinology, than if we start at the level of psychology.

Hammond. It seems to me—I want to refer to Professor Klein's remarks—that the ethical bar seems to be the trouble in applying the animal work to human beings. In domesticated animals we have a similar situation, but the bar is an economic one—cost—and the way we get over this is that before we study the costly animal we get some idea of its glandular complex and its reproductive pattern, and then we find a small laboratory animal with the same glandular pattern. We can then easily transfer the results on the small

animal to the larger one. What one wants is some animal with the human set-up. I would suggest, from the reproductive point of view, the monkey or the mare, which are very similar to man in their glandular complex and general set-up of reproduction pattern. I am not a psychologist, but I would propose that for this purpose you find some animal which has a mental pattern rather similar to the human being, on which you could test out your theories. From the domestic animal point of view I would suggest the pig.

Richter: I want to emphasize the importance of keeping good records of behaviour and all the factors that may influence behaviour. From such records it should be possible to establish a causal relationship between changes in behaviour and the various internal and environmental stimuli. Even in the simplest behaviour situations in animal and man the many variables that are present cannot be kept track of by ordinary methods of clinical note taking, it is necessary to have graphic records.

I can best illustrate this from my own personal experience with experimental studies on the self-selection of diets in rats. In these experiments each rat has access to a variety of purified food-substances in separate containers, 10 to 20 in all: dextrose, casein, olive oil, 5 minerals and 8 vitamin solutions. Daily records are made of the intake of each substance. We are interested in determining how the appetites for the various *substances change with relation to various experimental* interferences, such for instance as removal of the pancreas, the adrenals, the pituitary, or by pregnancy or lactation. It can readily be seen that it would be impossible to keep such a [illegible] make much sense out of

In this way even a quick perusal of a chart will establish the temporal inter-relationship between the operative procedure and the appetite for the various substances.

In studies on man it is equally important to have records that permit conclusions to be drawn regarding the temporal sequence of events.

I believe it is possible to use on human beings much the same graphic methods that have been worked out for animals.

Cleghorn: There are two points I would like to make, Mr. Chairman. One is the result of my attendance at a conference on psychosurgery in New York a year or so ago. Dr. Beach I think is familiar with the results of the psychological tests which were applied in the Greystone-Columbia project. A large battery of
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verifiable and definite differences in the patients after the brain operations. It was observed that there were two categories of people who could not carry on in their former professions: teachers and doctors. Apparently business men could go back, and even lawyers could make a living after these operations, but not doctors or teachers, in the practice of whose professions inter-personal relationships were rather important.

The second point that I wish to make is that science, as has been pointed out by Le Corbellier, has a very definite hierarchy: chemistry came before physiology, and physiology came before psychology. And we can see the recapitulation of this series of interests within present time. The interest, for example, in adrenal changes has been largely focused on the chemistry and physiology of the changes, so that those of us who were interested in these things a number of years ago were paying little or no attention to psychological factors, and we regret now that we did not have the psychological factors charted, as Dr. Richter has very ably pointed out that they should have been. We are perhaps only now making our beginning, and perhaps at another conference at a later date we shall be able to talk a little more precisely and a little less impressionistically than certainly I have been able to in this conference.

Beach: The experimentalist is primarily interested in the establishment of principles or laws of behaviour which he hopes will be applicable not only to rats and pigs but to human

beings as well. Because of this he is free to choose any one of many species to use as subjects in his experiments. The clinician, in contrast, is bound to the most difficult and complex species of all, and he is forced to be concerned with practical and specific cases. Any insight into general principles must come secondarily, however badly the clinician may want such information.

This sometimes leads to the conclusion that human behaviour is essentially chaotic, or at least infinitely more variable and complicated than is that of other species. However, practically everything that Dr. Reiss and others have said about the difficulties involved in clinical analysis could be applied also to the study of animal behaviour. In animals as well as in human beings, identical treatment often produces quite different results. But this simply tells us that there are still unidentified variables affecting our results. The number of such variables often can be reduced by experimental controls but their existence in all types of organisms is certain. The fundamental point here is that human behaviour is just *as lawful and just as much causally determined* as the behaviour of the rat or any other living creature. The mere fact that a multiplicity of factors are involved should not be discouraging—should not make us say that the clinical material is just too complicated to handle scientifically.

CHAIRMAN'S CLOSING REMARKS

S. ZUCKERMAN

THE conference has reached the point where the privilege of being Chairman assumes its most onerous aspect. I have to try to sum up. In doing so I shall indicate what I, more or less an outsider in this field, have gained from our discussions. You will have to forgive my presumption in attempting to present a comprehensive picture—and also any departures from the neutrality which is usually associated with a Chairman's functions.

Our central theme has been the endocrinological factors which underlie patterns of behaviour. In the main we have considered patterns that are innate, and only in one or two of the papers did acquired patterns come up for discussion—as, for example, in the contribution in which I raised the possibility that hormones can affect learning. The material of our discussion has been derived both from clinical observation and laboratory experiment, and in the latter part of our proceedings we found ourselves faced by the question of a possible fundamental difference in the scientific methods followed by the laboratory and clinical worker. The first point I propose making in my summing-up is that in my view any such difference, if indeed any exists at all, hardly impinges on fundamental questions of scientific methodology.

We have discussed different patterns of sexual activity in different species; but man, too, has a pattern of sexual activity, and can be included quite naturally in the same general category of discussion. We have considered whether different environmental factors affect patterns of endocrine action and patterns of behaviour. This, too, is a general question.

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made that emotional adjustment in the individual may affect

the balance of endocrine activity, not only through the adrenals, but also by way of the thyroid, as Dr. Reiss has suggested. We have also been trying to decide whether any specific alterations in social behaviour, aberrations of the kind that are defined as clinical entities by the psychiatrist, are associated with recognizable patterns of endocrine disturbance, and alternatively, whether different endocrinopathies are associated with specific psychological changes. This was a central theme in the contributions of Dr. Pincus, Dr. Reiss and Dr. Simpson. Professor Cleghorn provided us with an *analysis of the mental changes which take place in Addison's* disease, and Dr. Clifford Allen referred to adrenalectomy as a treatment for psychosis.

What we have been doing, on both the experimental and clinical side, is clarifying ideas in what is essentially a documentary phase of a science. Contrary, I imagine, to Dr. Beach, I do not believe that any of us has been very analytical. The word pre-Linnæan, which Dr. Beach suggested characterized our clinical contributions, does not seem to me to imply an appropriate differentiation between the clinical and experimental observations we have been considering—both are essentially constrained in the same way. For up to the present we have not really begun to explain one thing in terms of another, a pattern of behaviour in terms, for *example, of* an underlying web of neural connections—not at any rate at this conference. I therefore feel that while we have all gained a great deal of information from our various contributions, and that while we know a lot more than we did at the start about the possible articulation of endocrine factors with behavioural changes, we do not know how endocrine factors [illegible] That they do we can [illegible] Our general concern [illegible] both the clinicians [illegible] h a science that is [illegible] hed the stage when we can profitably argue about scientific methodology.

There seems to be a common feature to all the innate patterns of behaviour that we have been discussing. Whether it be a complicated pattern involved in courtship, or the depression of the Addisonian patient, we are dealing with

patterns of behaviour whose components are in the main separated, non-specific, but become spatially and temporally organized in some specific way. The behaviour of the stallion to which Dr. Walton referred; the behaviour of the hens and cocks in Dr. Domm's film; or the more complicated and prolonged behaviour of the roe deer which Dr. Hediger described, are all patterns of activity that have separate components which are spatially and temporally organized. It seemed in Dr. Domm's film as though all the features of the rooster's mating behaviour were common components of its ordinary life—pecking, walking around, stamping, and so on—and as though these various components were reassembled in a new totality significant to the particular situation of courtship. Apart from the final act of mating, there did not appear to be a single component of behaviour which was completely new.

Whatever be the way these separate components become articulated, from the neural point of view, into specific patterns of behaviour, we have learned quite definitely that these patterns are activated or powered by hormones. Here I need only remind you of the restoration of sexual responses and running behaviour in spayed and castrated animals, of the significance of adrenal function for maintaining normal levels of activity to which Dr. Richter drew our attention, and of the clinical observation, of which Professor Browne told us yesterday, that ACTH, as it were, revivifies the individual. In both cases, clinical and experimental, we are

experimental animal and the amount of hormone that is given, I have become much confused by the possibility that in human beings the same mental states can be associated with hyper-

states—and here I hope I do not misquote. Professor Cleghorn referred to patients suffering from Addison's disease,

individuals whose adrenal function was at a low ebb, as being very "depressed," "negative," "suspicious" and "tending to psychosis." Mr. Broster and Dr. Allen referred to patients suffering from adrenal tumours, and an excess of cortical secretion, as also being "depressed," "agitated," "hallucinated," and potentially psychotic. Dr. Pincus did not describe his schizophrenics, but I was under the impression that the same kind of verbal description also applies, at least occasionally, to them. A little earlier on Dr. Reiss said that the same syndromes, as designated by clinical diagnosis, may be associated with completely different patterns of hormonal excretion, and with very different patterns of thyroid activity. This rather confirms me in my view that the parallelism between states of behaviour and hormone balance is not as close in those human conditions we have been considering, as it is in the simpler patterns of sexual behaviour in animals that have been discussed.

A general idea that has emerged from our discussion, at any rate for me, is that many of the hormones we have been considering prime the whole organism. They prime it in such a way that a spatio-temporal pattern of neuromuscular responses can be triggered by some adequate stimulus. And it is fairly plain from the observations that have been discussed these past few days that certain neuromuscular patterns which in some sort of way depend on so-called "pathways" are identical in the two sexes; in one set of circumstances they become activated and in another dormant. But here again I personally come up against the obstacle to which I have already referred. Are the major endocrinopathies not necessarily associated with given psychiatric states, in the same way as a specific mental state, as we have heard again this morning, may not be tied to a definite endocrine picture? Have we to accept Dr. Reiss's thesis that these things are unpredictable because of "different constitutional factors?" I will refer to this matter and its scientific significance in a moment.

It is not just some internal drive which is primed and "fired" by differences in endocrine balance. As Mr. Eayrs emphasized in the second paper of the meeting, perception is also significantly affected by endocrine factors. This is a very

important fact. We want to know whether there is an adverse relationship between the strength of the stimulus and the

pugnacious male robin reacts sexually to the female robin, and that he also drives away other male robins from his territory As Lack has shown, what is responsible for his aggressiveness is just the sight of a few red feathers on the breast of a possible rival. There is a single significant feature in the total Gestalt. In this connection, I was much impressed by the last part of Dr. Domm's film, where the highly œstrogenized little hen,
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shown. What I should therefore like to know is whether different intensities of motivation can be associated with changes in the significant features of a total stimulus-pattern.

This leads me to the question of the existence or non-existence of the specific nervous pathways as being basic to the expression of the specific patterns of behaviour that we have been considering. Dr. Dempsey's observations indicated something in the nature of pathways, an observation which accords with older beliefs that afferent pathways from the generative tract are essential to such responses as ovulation. On the other hand, there are Professor Klein's observations, and those which Dr. Beach has brought forward, to the effect that afferent stimulation of the uterus and vagina is not essential for the release of specific patterns of sexual and maternal behaviour The question appears to be a very open one Our own discussions seem to have suggested that specific neuromuscular patterns are less important than the total reorganization, if one can use that word, of the activities of the organism. Again let me remind you of Dr. Domm's hens and roosters, in which drives were reorientated and reorganized according to the way their bodies were primed with sex hormones.

There are certain questions of fact to which I ought to draw your attention, matters which I hope will be cleared up in some later discussion, which the Ciba Foundation might

organize, say three years from now, when clinicians and experimentalists have drawn closer together in their mutual interests. I should like to learn more about the sequence of œstrogen-progesterone stimulation in the elicitation of histological, physiological, and behavioural changes, and of

Some observations, particularly those of Dr. Beach, seemed to indicate that certain behavioural responses could be used as indices in the assay of sex hormones, whereas others failed to indicate so close a relation. Another point which requires more investigation is the relation of emotional adjustment to the endocrine balance of the body, and here we need to know more about the effects of domestication. I should very much like to know whether the adrenal of the wild rat behaves like the adrenal of the domesticated rat; or whether its pattern of activity is different. Dr. Hammond, again drew our attention to the fact that we have not taken nutritional factors as much into account as we should have done. When we deal with conditions of extreme hypofunctioning of the endocrine organs, we are freq
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until we understand more than we do about innate patterns of behaviour it is going to prove very difficult to deal with the effect of various hormonal treatments on acquired behaviour.

The question of evolution was referred to a few times. The term needs to be used with a certain measure of circumspection. Evolution provides little justification for making
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to arrange behaviour patterns of the kind we have been discussing in an assumed evolutionary sequence, we have to remember that by so doing we return to a phase of zoology

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in behaviour does little to elucidate the way the evolution may have occurred. On the other hand, in so far as it is necessary to invoke the action of selection on a variable genetic system in order to explain the structural differentiation of organism, we may be sure that we shall have to call upon the same kind of process to interpret differentiations of

of the constitutional
cies differ from each
other—whether they be human beings, or rats, or blades of grass. In animal experiments we control the effects of such individual variation by means of properly-proved procedures. In clinical medicine we are inclined to refer to these individual differences as differences in constitutional factors. Now I believe that all experimental science is characterized by controlled observation and analysis The material we study is in some cases more amenable to this method than it is in others, and for the reasons that have been put forward at this meeting I am more than ready to believe that dealing with clinical material is infinitely more difficult than dealing with rats, dogs and monkeys. The essential problem, none-the-less, is how to control the factor of individual variability when

that in animal experimentation. Dr. Walton was right to remind us that statistical procedures have advanced considerably from the day when all one did was calculate the standard error of the mean or the variance, and compare one mean with another mean. We also have to remember that proper sampling is essential, and, as Dr. Kalmus has reminded us, it is equally essential not to impose statistical treatment as an afterthought on a lot of results, but to inject statistics into the general design of observations, whether they be clinical or experimental. If the diagnosis of various psychiatric disorders is as consistent as has been described, it should be possible to work with representative samples. And here I am

"true" in our field of study. What figures can do is to indicate when we are dealing with something which is likely to be in the category "true." I believe that in spite of all the difficulties that have been outlined, in spite of the need to keep the welfare of the patient uppermost in the clinician's consideration, that clinical material can be studied in a proper scientific way. And that is why I suspect Dr. Reiss's belief that the reason why the same results may not be obtained twice in the same individual, or in two different individuals, is because of the variability of constitutional factors. The same difficulty is encountered in animal work, but it can be overcome by proper experimental design and by adequate statistical treatment. Professor Browne suggested that even so, the most important variable might escape attention. That it may well do—but without these methods it is more likely that other important variables will tend to creep in, and obscure the whole picture.

Our discussions have focussed rather heavily on the adrenal glands. This is perhaps unfortunate, as I believe we have been dealing with activities in which many more physiological factors may be concerned. *But the original title of our* conference referred to steroid hormones; and so we were right to specialize the way we did. Our deliberations have, however, helped to underline the obvious fact that behaviour is certainly not controlled by hormones alone. To borrow a term which Dr. Beach used in his opening paper, we may say that in some species behaviour is still restrained by hormonal action, whereas in others, including ourselves, it has been emancipated from their strict control.

This conference has greatly widened our knowledge and experience. In the next that is organized on this subject, I trust that we sha [illegible]
of depth, and that [illegible]
of the way hormo[illegible]
system.

BOOK II

STEROID HORMONE ADMINISTRATION

RELATION BETWEEN EFFECT AND METHOD OF ADMINISTRATION OF ANDROGENS AND ŒSTROGENS TO FOWL

A. S. PARKES

THE work I am about to describe is all more than ten years old, but it provides a magnificent demonstration of the variation in length and intensity of effects which can be produced by administering hormones in different ways.

The action of a hormone is obviously observed most easily if the responding organ is externally visible, or at least externally accessible. Such an organ is especially useful where continuous observation of the effect is desired, to estimate the duration of effect of a particular hormone given in a particular way. Mammals are comparatively deficient in externally visible or accessible organs which respond to hormones concerned in reproduction. Domestic poultry, on the other hand, are very much better equipped. They have outstanding secondary sexual characters; the two most convenient ones being, of course, the comb and wattles, and the plumage. These two characters are under different control in domestic fowl. The comb is a simple dependent male secondary sexual character, and its size is dependent entirely on the activity of the testis in producing male hormone. The comb can easily be measured, and its behaviour under various treatments is therefore easy to assess. It is also ideally suited to the local administration of hormones and it has been much used in tests depending on the direct application of androgens and œstrogens. The comb has been used very largely in the assay of male hormone, and for investigating the effect of giving androgens by various routes and in various ways.

I want to talk on this occasion about plumage and its use for demonstrating hormone effects in the domestic fowl.

I shall restrict myself to the Brown Leghorn, a domestic fowl which has been much used for laboratory work because it shows extreme sex dimorphism in the plumage, notably in the ventral feathers which are black in the cock and fawn in the hen. The conditioning of this plumage type is well understood; castration does not affect the plumage of the male bird, whereas ovariectomy of the hen results in the appearance of male plumage at the next moult. Similarly, androgens are without influence on male plumage, whereas administration of œstrogens to the cock results in feminisation of the plumage. It is quite clear, therefore, that the male plumage is of the asexual or neutral type, and that the plumage of the female is conditioned by ovarian œstrogens. I should make it clear that existing feathers are not affected by any of these treatments, only those growing during or after treatment.

The essential features of feather growth are quite simple. The ordinary feather consists of a central rachis carrying a large number of barbs. As the feather grows each successive part is subjected to whatever hormone may be operating at the time, and the essential effects can easily be shown by giving a series of injections, the results obtained from other forms of administration being interpreted in the light of them.

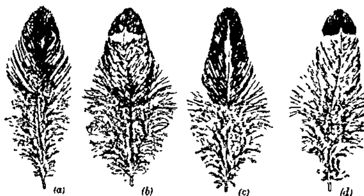


Fig. 1 (a)–(d) Diagram of types of response of breast feathers of Brown Leghorn capons to various types and doses of œstrogens (see text p. 250).

Sensitivity down the rachis of the feather is fairly constant, but the barbs show a sensitivity gradient from tip to base, the base being more sensitive to œstrogens than the tip. At any particular time a horizontal band of the feather is in course of development, so that sections of barbs showing different sensitivities are being laid down at the same time. The effects produced by œstrogens, therefore, vary with the concentration of the hormone at the time the feather is growing, as well as with the duration of its action. A short but intense stimulation will affect all parts of the feather growing over a short period, i.e. a large dose of an active form of œstrogen will convert the whole of a narrow band to the female colour (Fig. 1*b*). On the other hand, a short stimulus of low intensity converts only the more sensitive parts of the barbs, producing a spot on the rachis (Fig. 1*a*). Similarly, a heavy prolonged stimulus causes a [illegible] (Fig. 1*d*), [illegible] ing rachis [illegible]

This information can be used very elegantly to estimate the relative duration and intensity of effect of various esters of œstrogen. In the stock of capons at Mill Hill the average rate of feather growth was 2 mm. per day, so that the duration of effect can be found by measuring the part of the feather affected.

The four feathers in Fig. 2 show the results of doses of 100 µg, 250 µg, 1 mg. and 4 mg. of œstradiol. No increase in the duration of the effect was obtained by increasing the dose of the free hormone beyond a certain point; the maximum necessary intensity is produced by 1 mg. and no further increase of duration of effect is produced by raising the dose to 4 mg. Almost the same thing is seen with œstrone. While there is a slight increase in the width of the band by raising the dose from 250 µg to 1 mg., there is no increase by putting it up further to 4 mg. In other words, both of the free hormones are rapidly lost from the body. By giving œstradiol in the form of the monobenzoate, on the other hand, there is an increase in duration of effect with increased doses (Fig. 3), but the intensity is still adequate to affect the whole width of the feather. The comparative increase in duration of

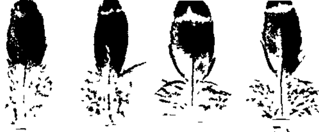


Fig. 2 Effect of œstradiol (left to right) 100 μg, 250 μg, 1 mg., 4 mg

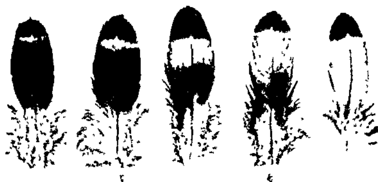


Fig 3. Effect of œstradiol monobenzoate (left to right) 50 μg, 100 μg, 250 μg, 1 mg, 4 mg



Fig. 4 (Left to right) Effect of 1 mg. œstrone as acetate, 1 mg œstradiol as benzoate, and 1 mg œstradiol as 3-benzoate-17-acetate.

Figs 1–4 from Parkes, *Biochem J*, 1937, 31, 579.

To face page 250]

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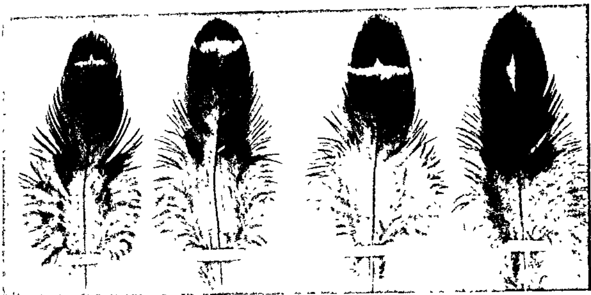


FIG. 5 (a) Effect of 250 μg. free œstradiol intramuscularly in 4 ml arachis oil (b) Effect of 1 mg free œstradiol intramuscularly in 4 ml arachis oil (c) Effect of 5 mg free œstradiol, intravenously in 1 ml propylene glycol. The duration of effect is similar to that produced by subcutaneous injection of a similar amount. (d) Effect of 250 μg. free œstradiol, inuncted to the *whole of one breast feather tract* in 1 ml arachis oil

FIG. [illegible]
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trat[illegible]

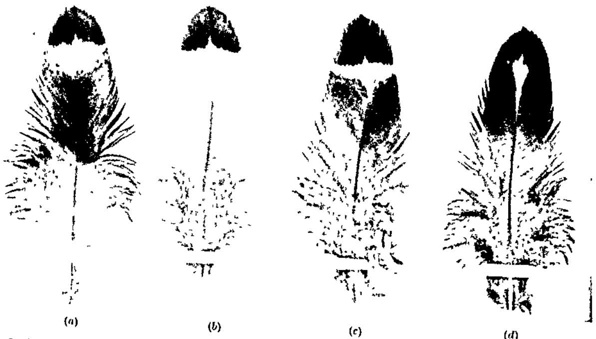


FIG. 6. (a) Effect of 250 μg. œstradiol-3-benzoate, intramuscularly in 4 ml. arachis oil. (b) Effect of 4 mg. œstradiol-3-benzoate, intramuscularly in 4 ml. arachis oil (c) Effect of 5 mg. œstradiol-3-benzoate, intravenously in 1 ml. propylene glycol (d) Effect of 250 μg. œstradiol-3-benzoate, uncted to the whole of one breast feather tract in 1 ml. arachis oil.





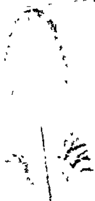




FIG. 7. (*a*) Lack of effect of 1 mg. œstradiol dibenzoate, intramuscularly in 4 ml. arachis oil. (*b*) Effect of 10 mg. œstradiol dibenzoate, intramuscularly in 4 ml. arachis oil. Prolonged stimulus of low intensity. (*c*) Effect of 20 mg. œstradiol dibenzoate, intramuscularly in 10 ml. arachis oil. Prolonged and intense stimulus. This feather began to grow immediately after the injection. (*d*) Effect of 2.5 mg. œstradiol dibenzoate, intravenously in 1 ml. propylene glycol. Much of the hormone was out of solution at time of injection.

threshold level required to produce even a trace of female colour. 10 mg., however, administered intramuscularly, gives a prolonged stimulus of low intensity. 20 mg. administered intramuscularly builds up not only a prolonged stimulation but also a fairly strong one, and as a result the whole feather is changed to the female colour.

The feather test gave birth to the pellet technique of administering the steroid hormones. We had certain reasons for believing that the duration of effect might be prolonged if the animal had considerable but not insuperable difficulty in absorbing the material, and we were therefore led to insert a large crystal of œstrone under the skin. I have said that 4 mg. of free œstrone injected intramuscularly in oil produced a band of female colour about 4 mm. across; that is to say produced an effect lasting perhaps a couple of days. However, when a 4 mg. crystal of free œstrone was implanted under the skin (Fig. 8) successive growths of new feathers for a total of 11 weeks became feminised, an effect about 40 times as long as that produced by injection of a similar amount of hormone.

In conclusion, I would like to emphasise that the plumage test is an extremely useful and practical one in work dealing with the duration of action of œstrogens given by various methods and by various routes. The œstrogen effect on plumage is only one of several that are due to hormone effects, and by using other breeds and other feather tracts it is possible to carry out the same kind of experiments with thyroid and other hormones. In considering material for new methods of biological assay for hormones the plumage of the domestic fowl should not be overlooked.

DISCUSSION

BROWNLEE Has this method ever been applied to adrenal cortical hormones?

PARKES So far as I know, it has not been applied to adrenal cortical hormones.

BROOM: What is the variation between feathers?

[illegible]

from feather to feather, where the response is remarkably uniform.

BROOM: Can you give hormones orally to the fowl? Is there quick absorption from the fowl stomach?

PARKES: Stilbœstrol is active by mouth.

BROOM: How does the relative potency of œstrogens in the fowl compare, say, with the rat?

PARKES: The serial variation of the aliphatic esters of œstradiol is the same in the fowl as in the rat.

GADDUM: Has the test been used by other people?

PARKES: Greenwood in Edinburgh has used it quite a lot, though not from this particular point of view.

BROWNLEE: Can it be used in smaller birds, the pigeon for example?

PARKES. There is at least one kind of bantam where the plumage dimorphism is of the same type. Pigeons do not show sex dimorphism in plumage.

FOLLEY: Does this sexual dimorphism apply to other species? We once had two peahens and thought we would try to turn them into peacocks. Unfortunately we knew very little about the theory of plumage in birds and made the mistake of not consulting Parkes about this beforehand. We put testosterone pellets into the peahens but without much effect. Would it have been possible to have done it by ovariectomy?

PARKES: Yes.

BISHOP. Have you investigated the stilbene œstrogens with this technique, and if so, how did they compare in potency?

PARKES. I think Greenwood did that for Dodds some years ago. The original, rather inactive, artificial œstrogens were tested on capons.

BISHOP: Do you happen to know what the order of potency of the stilbene group was?

PARKES: No.

I ought perhaps to say that in all ordinary domestic poultry the dimorphism is as described here, that is, the female has female plumage because she has an ovary; the male has male plumage because he hasn't got an ovary. Where there is no colour dimorphism, there is the usual dimorphism in the shape of the feather. There are one or two fancy breeds of bantams in which the male also has female plumage, due to abnormal activity on the part of the testis or to abnormal sensitivity of the plumage. On the other hand there are some African weaver finches, investigated by American workers, in which certain types of plumage are conditioned by the gonadotrophic hormones of the pituitary.

GADDUM: Do the androgens have any effect on plumage in any species?

PARKES: Only where the cock has hen feathering.

FORBES. Androgens will cause male colouration at the base of the bill of the sparrow. It's been used to some extent in the assay of male hormone. It is a sensitive test, responding to one microgram of testosterone.

PARKES: There are several simple dependent characters of that kind. For instance, in one species the leg colouring is governed by androgens.

threshold level required to produce even a trace of female colour. 10 mg., however, administered intramuscularly, gives a prolonged stimulus of low intensity. 20 mg. administered intramuscularly builds up not only a prolonged stimulation but also a fairly strong one, and as a result the whole feather is changed to the female colour.

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DISCUSSION

BROWNLEE: Has this method ever been applied to adrenal cortical hormones?

PARKES: So far as I know, it has not been applied to adrenal cortical hormones.

BROOM: What is the variation between feathers?

PARKES: There is the same order of variation as might be found in any other test. The feathers shown were in each case all from the same bird, but groups of 5 to 10 birds were used always The variation from bird to bird, as you would expect, is very much greater than from feather to feather, where the response is remarkably uniform.

can be compared in activity with crystal suspensions. The first experiment was performed with testosterone propionate. White leghorn capons were injected with 5 mg. of testosterone propionate in oily solution, as a crystal suspension, and as an emulsion, and Fig. 1 shows the comb growth which occurred in each instance.

The next experiment was performed with œstradiol monobenzoate. Castrated female rats were injected and the duration of œstrus was determined. Table I shows you the

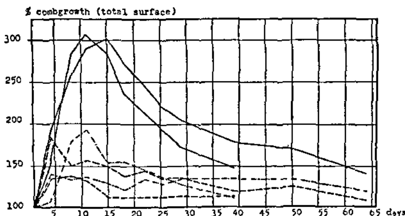


FIG. 1. Comb growth after one single intramuscular injection of 5 mg. testosterone propionate into capons. Each group consists of six animals.

———— Emulsion.
— — — — Oil solution.
–·–·–·–· Crystal suspension prepared *ad hoc* in the 64 days test. A commercial crystal suspension prouct in the 39 days test.

comparison of the effect obtained with the oily solution, the crystal suspension and the emulsions. Œstrus lasted for 12 days with emulsions and the suspensions, and only for 5 days with the oily solution. From this experiment it would seem that the emulsions and the suspensions have almost equal activity, but there is one difference. When full œstrus had disappeared in practically all rats, it turned out that the animals which got the suspension remained in

SOME DATA ON EMULSIONS OF STEROID HORMONES

G. A. OVERBEEK

USUALLY the steroid hormones are administered as solutions in oil. You all know the practical disadvantages of this method of administration. After implantation of tablets there came crystal suspensions, which have been of much use though they are rather difficult to handle. This method has been supplanted by that of Zondek in which the crystals form in the syringe, and now Dr. Lens and Dr. Polderman have brought the process one step further by making the crystals form in the tissue itself. The idea was to find a solvent for the hormone which is almost immiscible with water but which is more soluble in water than the steroid itself. Furthermore, it should be a very good solvent for the hormone. After injection of such emulsions into the tissues, which is equivalent to mixing with large quantities of water, crystallization of the hormone should occur. The solvent should be non-volatile because it must be capable of being sterilized, and it must not be toxic. There are very few solvents which fulfil all these requirements, but we find benzyl alcohol very suitable. It is possible to make very highly concentrated solutions of various steroid hormones in it. A solution of the steroids is mixed in a Waring blender with water containing a glycol buffer and glucose to make it isotonic. In certain instances, for example with the œstrogenic hormones, it is necessary to add some salol in order to lower the melting point of the steroids further than is necessary with other compounds.

These emulsions have a milky appearance and are very stable. Only after long standing do they tend to break up; then simple shaking is enough to produce an emulsion which will last for weeks.

I shall describe some biological experiments which show that these emulsions have a prolonged action and that they

the effect of the suspension the feathers were not evenly brown but mottled. You will remember that I told you that in the œstrus experiments the rats treated with the suspension remained in metœstrus. Probably once a definite effect is established very small quantities of the œstrogens suffice for these small but long-lasting after effects. This was not observed either with testosterone propionate or with deoxycorticosterone acetate (D.C.A.).

To compare the effects of D.C.A. we used the survival time of adrenalectomized young rats. The animals were injected on the day of operation and one week later, both times with 5 mg. D.C.A. The following table II shows the mean survival times with the standard errors of the mean.

Table II

Preparation	Number of rats	Survival time in days
Emulsion . . .	16	28.4 ± 1.01 (1)
Suspension . .	15	27.4 ± 2.28 (2)
Oily solution . .	20	17.4 ± 1.40 (3)
Not injected . .	14	10.1 ± 0.92 (4)
Difference (1)-(3) .	t_{34} = 6.38 P	<0 001
,, (2)-(3) .	t_{33} = 3.50 P	<0.01
,, (3)-(4) .	t_{32} = 4.35 P	<0.001
,, (1)-(2) .	not significant.	

Apparently the effects of the emulsion and the suspension are comparable. However, the standard error in the group treated with the suspension is much higher. Both groups caused a significantly prolonged survival, compared with the oily solution.

To conclude the animal experiments I wish to say just a few remarks on the toxicity. Rats were injected subcutaneously with 0.25 ml. of the emulsion for a period of 6 weeks. This treatment was tolerated without loss of weight or any other ill effects other than some hardening of the skin. Other experiments with frogs and dogs showed the same low toxicity. The local effects, as shown by subconjunctival injection into rabbits, were small.

metœstrus for a longer time than the animals which were given the emulsion. There is a difference of many weeks. Therefore it seemed that in the animals which received the suspension traces of the hormone remained for a very much longer time than in those which had received the emulsion. Similar results were found in the discolouration of the feathers of the Brown Leghorn capon, the test described by Dr. Parkes earlier at this meeting. Breast feathers of capons were plucked and the new feathers were studied.

Table I

THE ŒSTROGENIC EFFECT OF ŒSTRADIOL MONOBENZOATE IN DIFFERENT MEDIA IN CASTRATED RATS

Total dose in all series 0·5 mg. As a comparison œstradiol crystals are included.

No of experiment	Preparation	No of animals	Duration of œstrus in days	Remarks
I	emulsion	6	12	
	crystal suspension	6	12	Organon manufacture
	solution in oil	6	5	
	œstradiol crystals	6	6	
II	emulsion	12	7	
	crystal suspension	12	4	commercial product
	solution in oil	11	4	
	diethylstilbœstrol-emulsion	11	3	

Three groups of six capons each received one intramuscular injection of 10 mg. œstradiol benzoate either as an emulsion, a crystal suspension or an oily solution. As expected, discolouration started at the base of the feathers and the time was observed during which this part of the feather was brown. The mean duration of the effect was 15, 56 and 9 days respectively. This shows again the prolonged effect of the emulsion as compared with the oily solution and also the much longer lasting effect of the suspension. The result is completely comparable with the result of the already mentioned œstrus experiments, as during the latter phase of

Furth [illegible]
with a [illegible]
benzoate, [illegible]

I hope I have been able to show you in a convincing way that these emulsions can be very effective. However, there is one difficulty with these emulsions: the pain after injection. This is rather remarkable, because pain occurs not at the moment of injection but only after 4–6 hours, and the site of the injection or even the whole leg may remain painful for several days. This has been found only with testosterone propionate and not with the other hormones, and even with testosterone propionate many injections are painless.* I do not know what is the reason for this pain. It cannot be the benzyl alcohol or any other constituent of the emulsion, for in that case the injection of D.C.A. or the other hormones or control injections would also be painful, and they are not. It may have something to do with the size of the crystals. We must solve this problem. The addition of local anæsthetics would be of no use at all, because the painfulness appears only after an interval and is then of long duration. However, apart from this one disadvantage I think the emulsions should prove very useful.

DISCUSSION

FOSS: How does the viscosity of the emulsions compare, for example, with solutions of testosterone propionate in arachis oil?

OVERBEEK: The viscosity is very low; they are like water. They are very easily injected and the syringe can be cleaned very easily. [illegible]

OVERBEEK: Yes, the crystallization occurs in the tissues; the benzyl alcohol diffuses away and leaves only the crystals

GROSS: May I complete Dr. Overbeek's remarks on the subject of pre-formed crystals? The duration of action depends on two factors: the first is the size of the crystals and the second is the solubility of the crystal injected.

Experiments performed by Tschopp in our laboratories showed that the duration of action was longer with pre-formed crystals than with

*It has since been found that somewhat lower concentrations, i e. 25 mg /ml are not painful.

But probably you will be more interested in the results obtained in man. Unfortunately not much material is available as yet and very often it is difficult to obtain the quantitative data which are necessary to compare the different modes of administration.

On testosterone propionate I cannot give you any useful information. From Dr. Férin (of Liége) we received some nice data on the effect of œstradiol benzoate in 3 castrated women and one woman with ovarian agenesis. The latent period elapsing between the second injection of 10 mg. (a

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Other experiments of Dr. Férin were on the occurrence of hot flushes in hysterectomised women. In one case after injection of 10 mg. of an emulsion of œstradiol benzoate hot flushes remained absent for 70 days; in two other cases 25 and 20 days respectively. Then there are some experiments with D.C.A. which was given to patients with Addison's disease. Most of the results are from the work of Dr. Ernould, another Belgian physician, who gave a total of about 50 injections of 50 mg. in four cases of Addison's disease. He concluded that there is no sparing action. By "sparing action" I mean that less hormone is required, e.g. if such a patient needs 5 mg. a day, one injection of 50 mg. of the emulsion would not last for 10 days, but for 20 days. However, when I looked over his results I thought that there might still be some sparing action. Even if this is not so, it is clear that it must be much more agreeable for a patient to receive one injection every 10 days instead of daily. There are some other clinical results: in one patient who needed 5 mg. daily, one injection of 50 mg. of the emulsion was active for 12–13 days. In another patient a comparison was made between emulsion, crystal suspension and implantation

...s; 50 mg.

ntation of

emulsions

plantation

pellets.

which the hormone is given. You can find giant cells and later the production of granulomatous and connective tissue.

FOLLEY: How do you keep your pre-formed crystal suspensions stable?

GROSS: Substances like methyl cellulose are added to give higher viscosity to the suspension. They are shaken before use.

MALPRESS: In regard to the sterilizing of œstrone solutions, if we heat an oily solution to 100° C., are we running any risk of diminishing the activity of the hormone? If these oily solutions are heated to body temperature, is there any diminution of activity?

BROWNLEE: Several years ago I showed that œstradiol, œstrone and diethyl-stilbœstrol were fairly strongly bactericidal.

GADDUM: Are we to assume that we need not sterilize these solutions at all, but can rely on the hormone's bactericidal powers?

BROWNLEE. Could Dr. Gross tell me whether there is an optimal size of crystal?

GROSS: Yes, there is an optimum relation between the surface of

arthritis, and we are anxious to inject very large amounts of these hormones and to alternate our administration of the steroid substance with a course of administration of some inert substance. We have

have any experience of this.

OVERBEEK I am not sure that it is possible to make an emulsion of cortisone It could be done with progesterone. It is difficult to say how long the effect of an injection of progesterone emulsion lasts. There may be something in the possibility of using injections of molecular size crystals. An emulsion is certainly a way of injecting large doses in a very small volume.

BISHOP: But then you would get the prolonged effect, which is what we are seeking to avoid

PARKES: Could you not give the steroid intravenously? If you want a short sharp action, surely that would be the way to do it?

BISHOP There are practical difficulties. Most of our rheumatoid arthritic patients have very bad veins.

DEANESLY: Could you not put in a batch of small tablets and take them out again?

emulsions. An explanation for the difference in these results from those of Dr. Overbeek might be that the emulsions, after injection, do not always form crystals of the same size in the tissues.

Another important factor for the duration of action is the solubility of different esters of testosterone or other steroid hormones. For example, testosterone isobutyrate, an ester which is less soluble than testosterone propionate, shows nearly the same duration of action with crystals of 0.1 mm diameter as testosterone propionate crystals of 0.15–0.3 mm. diameter. Using the same ester, the duration of effect is also seen to be dependent on the size of the crystals, increasing with increased size. In each case, of course, the total quantity of the hormone was the same. The difference between our results and those of Dr. Overbeek may be due to the difference in the size of the pre-formed crystals.

FOLLEY. What effect had the implantation of a 10 mg. tablet, compared with your crystal suspensions?

GROSS: With testosterone propionate the duration of action would be much longer with the tablets. However, with testosterone iso- [illegible]

a preparation of the hormone in aluminium phosphate solution, and this preparation in turn had a more prolonged effect than an oily solution. However, a suspension of pre-formed crystals of very small diameter showed a shorter action, like the oily solution.

We have also measured the daily absorption from a depot of pre-formed crystals. If you inject sufficient hormone to raise the amount absorbed to twice the threshold level, the level will stay up for about 6 days before falling to the threshold level, but if you inject twice this dose you will have an action of only 10 days. Therefore there is an [illegible]

need further study.

KLEIN: Did you make any histological examination of the sites of injection?

GROSS: Histological examinations have been made, and the appearances depend on the hormone and the suspension medium in

DATA ON RELATIVE ABSORPTION RATES OF SUBCUTANEOUS PELLETS OF STEROID HORMONES IN RATS

T. R. FORBES

I INTEND to make this report brief as the work is not very recent, has been published, and is probably familiar to most of you. The question I attempted to answer was, what are the relative absorption rates of pellets of various hormones in rats? In planning the experiment, the attempt was made to eliminate all other variables so as to make a strict comparison between the hormones. The pellets were cylindrical; we attempted to make them uniform by always using the same weight for compression. (This may have been unnecessary; it is certainly unnecessary now when one can make pellets by fusion.) We implanted 10 mg. pellets subcutaneously under the flank skin of adult rats. The animals were anæsthetized so that they would not struggle and possibly break the pellets. A pellet was taken up in the lumen of a large hypodermic needle, the needle was thrust under the skin, and the pellet was discharged by pushing the plunger. The pellets, of course, had been carefully weighed before implantation. At intervals the pellets were removed, dried, and re-weighed. It was possible then to determine the percentage loss of weight, and the latter could be plotted against time. Curves were obtained up to 90 per cent absorption. When the pellets were examined under the microscope it was seen that their surfaces remained smooth.

We followed exactly the same procedure in regard to each of the hormones and obtained a whole series of curves. It should be remembered that these absorption rates are not absolute; they relate to these particular compounds, administered in this particular way, in these particular rats. The number of days required to obtain 90 per cent absorption was determined for each hormone. The most rapidly

BISHOP: Yes, but it is a nuisance to take them out of every patient after a fortnight. However we may be driven to doing it that way. There is also, particularly with progesterone, the tendency to extrusion.

GROSS: There is the same difficulty with pregnenolone. We had many complaints when we gave oily solutions of it, but when we used a fine crystalline suspension in saline, the local tolerance was much better. Oral administration should be considered.

BROOM: Did you try more than one oil?

BISHOP: We tried ethyl oleate, which is a pure oil, unlike arachis oil, which is a mixture. I have no doubt that it is the quantity of oil that is important, because with 1 or 2 ml. we get no difficulty, but with 3 ml. we get the most intense pain.

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We followed exactly the same procedure in regard to each of the hormones and obtained a whole series of curves. It should be remembered that these absorption rates are not absolute; they relate to these particular compounds, administered in this particular way, in these particular rats. *The number of days required to obtain 90* per cent absorption was determined for each hormone. The most rapidly

absorbed compound was deoxycorticosterone, 90 per cent of which was absorbed in 27 days; with testosterone, the time was 31 days; with methyl testosterone, 36 days; diethylstilbœstrol, 51 days; hexœstrol, 54 days; testosterone mono-propionate, 61 days—an example of the fact that esterification leads to prolongation of action; deoxycorticosterone acetate, 64 days; progesterone, 88 days; œstriol, 93 days; androsterone, 120 days; diethylstilbœstrol dipropionate, 140 days; alpha-œstradiol, 180 days; alpha-œstradiol dipropionate, 220 days; testosterone dipropionate, 272 days; œstrone, probably 400 days; androhydroxyprogesterone was exceedingly slow—less than 30 per cent was absorbed in 300 days. Cholesterol was not absorbed at all.

The pellets were found to be enclosed in layers of connective tissue, which were pretty well formed within a week. Occasionally there was evidence of infection. In two cases the thickened capsule seemed to have accelerated absorption. What this accelerated absorption means, I am not sure. Perhaps the blood supply was better in some cases of thickened capsule and worse than the average in others. There were some side effects noticed during this work, for example changes in the hair and development of mammary glands in pre-puberal and post-puberal rats when testosterone propionate was administered.

STUDIES ON THE ABSORPTION OF PELLETS OF STEROID HORMONES AND RELATED SUBSTANCES IN MAN

P. M. F. BISHOP and S. J. FOLLEY

Dr. Folley

This is a report of the results of work carried out between 1942 and 1944 on the rates of absorption of pellets of various hormones and related substances* implanted into human beings. Data on pellet absorption in man, published up to the present time, are rather scanty and the investigation reported here is, as far as we are aware, the most extensive yet carried out. It is therefore hoped that these results may be of practical use as regards the clinical administration of hormones.

The tablets were specially made for this work, no diluent or excipient being used. Cast or fused pellets were cylindrical in shape, the height being somewhat greater than the diameter; the compressed pellets were cylinders with convex ends. The study embraced nine hormones or hormone derivatives (esters) and wherever possible the absorption of fused or cast pellets was compared with that of compressed pellets of the same substance. This comparison could not be made for the synthetic œstrogens related to stilbene, as, in our experience, cast pellets of these, implanted both in human beings and animals, tend to break up soon after implantation, giving a "mush" of hormone in the implantation site. Therefore, in the case of stilbœstrol and hexœstrol we have results only for compressed pellets.

The pellets as received from the makers were dried to constant weight in a desiccator over $CaCl_2$, weighed and

*For brevity the term "hormone" is used hereafter [illegible]

hormonal activity

measured. They were then returned for sterilization to the factory, from which they were finally sent to us packed ready for use.

Implantations were made into patients of both sexes according to their needs. The technique has been described by Bishop (1949). Whenever possible the implantation was subcutaneous, but in some cases, notably progesterone, pellets of which tend to extrude easily (see Bishop, 1949), it was necessary to implant them more deeply. Where the chance of extrusion was considered to be small, the implantation was made subcutaneously in the arm or hip region. The pellets were removed at various times after implantation, and for each substance we endeavoured to contrive a fairly uniform and widespread distribution of implantation periods so as to define the absorption curve as well as possible. After removal, the tablets were carefully cleaned, washed, and dried to a constant weight. The hormone was then dissolved in ether in order to obtain information on " ghost " formation, that is, the extent of deposition of insoluble protein material from the host in the interstices of the tablet (Folley, 1944). With compressed tablets implanted into experimental animals, voluminous " ghosts ", which retain more or less the shape and general form of the tablet after the hormone has been carefully extracted with organic solvents, are uniformly obtained. Cast pellets, however, which presumably do not have such large pores or interstices, do not deposit " ghosts ", though surface membranes (of negligible weight) are often formed (Deanesly and Parkes, 1943). In the present investigation " ghosts " of the usual type were deposited in most but not all of the compressed pellets and surface membranes were formed round the cast pellets.

Androgens

Testosterone. The absorption curve for cast pellets of testosterone, weighing approximately 100 mg., implanted into men is shown in Figure 1. This is the only section of these results which has been published previously (Bishop and Folley, 1944). A former colleague of one of us, Dr. A. C. Bottomley, kindly deduced for us an equation for the absorption rate of a cylindrical pellet on the assumptions (*a*) that

at any time subsequent to implantation the rate of absorption is proportional to the area of the cylinder at that time, and (*b*) that as absorption proceeds the tablet shrinks uniformly in all dimensions. The unbroken line in Figure 1 is

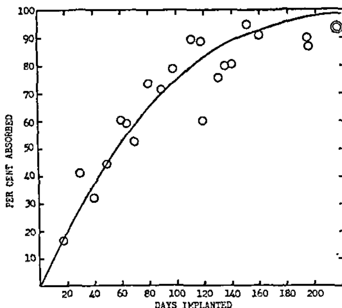


FIG 1. Absorption of cast pellets (100 mg approx.) of testosterone implanted into men The unbroken line is plotted from the equation —

$$A = 100\left\{1 - \frac{(r-kt)^2(h-2kt)}{r^2h}\right\}$$

the theoretical curve calculated from this equation for cylindrical pellets of the dimensions used by us. It will be seen that the data fit remarkably well to this theoretical curve, considering the technical difficulties inseparable from an investigation of this sort, and it may be assumed that cast, cylindrical pellets of testosterone are absorbed at a

rate which can be predicted from the dimensions of the pellet with reasonable accuracy.

Figure 2 shows the absorption data for compressed pellets of testosterone weighing approximately 100 mg. Here the spread of the results is much larger than with the cast pellets and therefore we have not attempted to fit a theoretical curve to the data as in Figure 1, although an absorption equation could be deduced for cylinders with convex ends which was the shape of all the compressed pellets used in these studies. In order to obtain for the compressed pellets an approximate

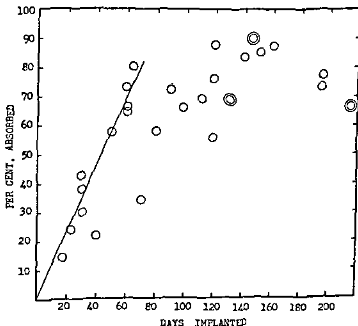


Fig. 2. Absorption of compressed pellets (100 mg approx.) of testosterone implanted into men. The initial absorption rate estimated graphically = 1·2 mg./day.

value for the initial absorption rate which, in the case of the cast pellets could be calculated from the equation (see Cowie and Folley, 1946), we drew by eye the best-fitting straight line through the points up to 50 days, which seemed to fall more or less on a straight line. From this set of results it

may be concluded that the absorption rate of compressed pellets of testosterone was less uniform and less predictable than that of cast pellets and, further, that the rates of absorption of cast and compressed pellets of testosterone of comparable weight (though differing in surface area by about 10 per cent) are not widely different. Nevertheless, it seems permissible to conclude from our results that in the case of testosterone, cast pellets are preferable to compressed ones because they give a more uniform and predictable rate of hormone uptake.

Testosterone propionate. Figure 3 shows absorption data for cast and compressed pellets (100 mg. approx.) of

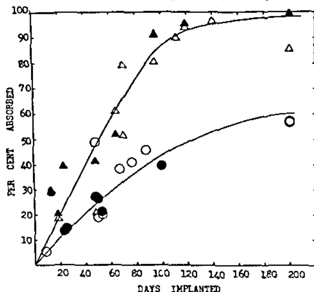


FIG 3. Absorption of pellets (100 mg. approx.) of testosterone propionate and androstendione implanted into human beings.

○ Testosterone propionate (compressed pellets)
● Testosterone propionate (cast pellets)
} Initial absorption rate, estimated graphically = 0·6 mg./day

△ Androstendione (compressed pellets)
▲ Androstendione (cast pellets)
} Initial absorption rate, estimated graphically = 1·0 mg./day.

testosterone propionate. The results for both types of pellet fall very nicely along the unbroken curve which was drawn by eye, indicating that there is no appreciable difference in the absorption of the two types of pellet in the case of testosterone propionate. Moreover the absorption rate of the ester is much smaller than that of free testosterone in agreement with the results of Deanesly and Parkes (1937) and Emmens (1941) in the rat.

Androstenedione. The results for compressed and cast pellets (100 mg. approx.) of androstenedione are also shown in Figure 3. Again the results for pellets made by both methods seem to fall along the same curve which once more has been drawn by eye. Undoubtedly androstenedione is absorbed much more quickly than testosterone propionate; our results indicate very little difference between the absorption rate of free testosterone and androstenedione.

Methyltestosterone. Only two determinations were made with methyltestosterone pellets (cast). These are shown in Figure 8, and suggest that methyltestosterone is absorbed at a rate comparable with those of testosterone and androstenedione and apparently faster than testosterone propionate.

Œstrogens

Œstradiol. Absorption curves for cast (100 mg. approx.) and compressed pellets of œstradiol respectively are shown in Figure 4. Two sizes of compressed pellet, weighing approximately 100 mg. and 50 mg., were investigated. The absorption data for both sizes of tablet have been plotted on the same graph, this being possible because, as in all cases, the per cent of the original tablet absorbed has been plotted against time. Here again the variance of the results both for cast and compressed pellets is somewhat greater than desirable. Nevertheless the initial absorption rates read from curves fitted, as before, by eye, show quite clearly that the absorption rate of œstradiol is much slower than that of any of the androgens studied. Even at 240 days the pellets were only about 50 per cent absorbed whereas testosterone pellets were 90 per cent absorbed in about 140 days. With compressed pellets the values for the per cent absorption of pellets of both sizes fell on the same curve (or perhaps it

would be better to say, fell within the same zone) and moreover there was no apparent difference between the absorption rates of the cast as compared with the compressed tablets. The reason for the marked variability of the results obtained with pellets of this œstrogen is not known.

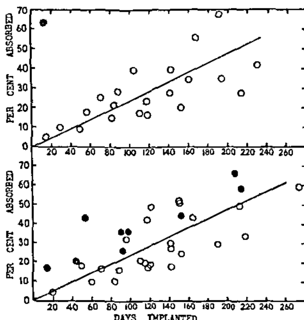


FIG. 4. Absorption of pellets of œstradiol implanted into women.

Upper curve, cast pellets weighing 100 mg approx. The initial absorption rate, estimated graphically = 0 24 mg./day

Lower curve, compressed pellets —
○ 100 mg approx.
● 50 mg. approx

The initial absorption rate, estimated graphically = 0 24 per cent /day.

Œstradiol dipropionate. Figure 5 shows the results for 100 mg. (approx.) compressed pellets of œstradiol dipropionate (curve fitted by eye). Here again the absorption rate is low compared with that of the androgens and scarcely differs from that of free œstradiol. If anything, our results

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œstradiol would be preferable where a slow absorption of small daily doses of œstrogen over a long period is needed. As can be seen from the absorption curve for œstradiol pellets (Figure 4), absorption proceeded at a fairly steady (though, in the case of any one tablet, not very closely predictable) rate for a period of nine months to a year, whereas stilbœstrol tablets were about 80 per cent absorbed in about 150 days.

Hexœstrol. The absorption of compressed pellets of hexœstrol is shown in Figure 7. In this case also pellets weighing approximately 100 mg. and 50 mg. were studied. The results for both sizes fall nicely along the same curve,

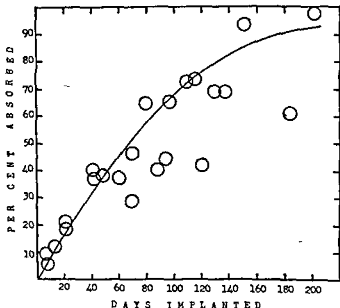


FIG 6. Absorption of compressed pellets (100 mg. approx.) of stilbœstrol implanted into human beings The initial absorption rate, estimated graphically = 1·0 mg /day.

fitted by eye, and from the initial absorption rates read from the curves for the two stilbene derivatives it may be concluded that in man there is no appreciable difference between the absorption rates of hexœstrol and stilbœstrol. This result

indicate that the dipropionate is absorbed slightly faster than the free hormone (see Table I). This is an interesting result, because it is generally assumed, and it is certainly the case with testosterone, that esterification of steroids with propionic acid appreciably slows up absorption. However, it is worth noting that Emmens (1941) reported exactly the same phenomenon in the rat.

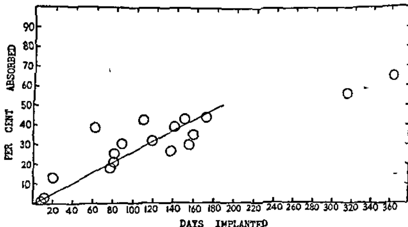


FIG 5 Absorption of compressed pellets (100 mg. approx.) of œstradiol dipropionate implanted into women. The initial absorption rate, estimated graphically=0 26 mg /day.

Stilbœstrol. The absorption data for 100 mg. (approx.) compressed pellets of stilbœstrol are shown in Figure 6. Most of the results fall satisfactorily on the usual type of curve rather similar to the curves obtained with androgens. The curve shown in Figure 6 was drawn by eye. It is evident that stilbœstrol pellets are absorbed much more quickly than pellets of œstradiol, so that for purposes necessitating the fairly rapid absorption of implanted pellets, perhaps even to completion (e.g. in order to obviate the removal of un-

œstradiol, other factors being equal. On the other hand,

in Figure 8. The available data seem to fall on a regular curve.

Anhydro-hydroxy-progesterone (Ethinyl Testosterone, Ethisterone). Two values for the absorption of 100 mg. (approx.) *compressed pellets* of anhydro-hydroxy-progesterone are shown in Figure 8. In agreement with

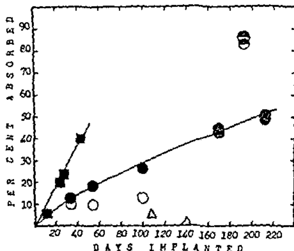


FIG. 8 Absorption of steroid pellets implanted into human beings

○ [illegible]
● [illegible]
■ [illegible]
day.
◓ Fused methyltestosterone; approx. 100 mg.
△ Compressed ethisterone; approx. 100 mg

previous workers *we find practically no detectable absorption* of this substance within any reasonable time.

Deoxycorticosterone Acetate. Figure 8 shows the results obtained with 100 mg. (approx.) cast and compressed pellets of deoxycorticosterone acetate. The results for the *cast pellets fall on a smooth curve of the usual type.* *There* are very few results for compressed pellets but as far as they go they do seem to indicate that absorption of compressed

is different from that obtained previously in the laboratory of one of us, in experiments in which somewhat larger pellets, weighing 1 g., were implanted into cows or heifers (Folley and Malpress, 1944). In these latter experiments, the cow absorbed stilbœstrol at a *significantly greater speed* than hexœstrol; this apparently is not the case in man.

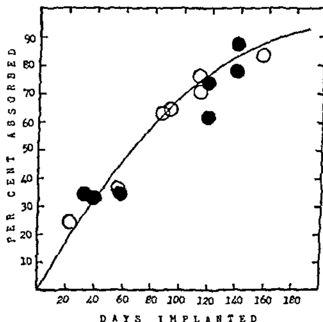


FIG. 7. Absorption of compressed pellets of hexœstrol implanted into human beings.
○ 100 mg. approx.
● 50 mg. approx.
The initial absorption rate, estimated graphically = 1·0 per cent/day.

Progestogens and Deoxycorticosterone

Progesterone. We have comparatively few results for *pellets of progesterone* because if these are implanted *in a position* sufficiently superficial to permit of recovery they are almost always extruded. Such results as we have with 50 mg. (approx.) cast pellets of progesterone are presented

mathematical methods. The values are thus to be regarded as only approximate and are not necessarily the most probable values statistically, but they can be considered as being as near to these as it is worth while trying to go considering the magnitude of the experimental error.

Pellets of the various substances studied have been tabulated in descending order of rapidity of absorption as far as this could be ascertained with the approximate methods used but it must be remembered that owing to the experimental error this order is not certain among substances which do not differ much in absorption rate. The Table is completed by results on man taken from the paper of Foss (1943), by results for the rat taken from Emmens (1943) and recalculated in terms of per cent absorption per day, and by the results of Forbes (1943) for the rat which he expressed in terms of the time taken for 90 per cent absorption. It was not possible to recalculate Forbes' results to a basis which would permit of direct quantitative comparison with our own results but it is possible to compare the relative rates of absorption of various hormones as found by Forbes and ourselves respectively.

It will be seen that the substances investigated by us fall broadly into three classes as regards their absorption rates when implanted into human beings in the form of pellets made without diluent. The first class comprises hormones which are fairly rapidly absorbed and all at much the same rate within experimental error—testosterone, androstenedione, progesterone, hexœstrol and stilbœstrol. The second class consists of hormones absorbed relatively slowly, at about 25 per cent of the rate of the former group, and includes œstradiol dipropionate, deoxycorticosterone acetate, and œstradiol. Intermediate between these is the third group, consisting of one substance only, testosterone propionate, absorbed at about half the rate of the rapidly absorbed hormones. There appears to be no significant difference between the rates at which cast and compressed pellets of any given substance disappear. The results of Foss (1942) and Emmens (1941) on man and the rat respectively show fair agreement with ours. The relative order of rapidity of absorption given by Forbes (1943) differs from our order in certain respects, the most noteworthy being the relatively

pellets of deoxycorticosterone acetate is slower in man than that of fused pellets.

Summary of Results

Our results are summarised in Table I in which are also given for comparison results for the rat as well as man reported by three other investigators. Our results are expressed not only as initial absorption rates (in terms of per cent of the original pellet absorbed per day), but also as the half-lives of the pellets. Both were estimated from the absorption curves, which were fitted to the data by eye except in the case of Figure 1, where a theoretical equation was fitted by

Table I

Data on the Absorption Rates of Hormone Pellets in Man and the Rat

	Man		Man	Rat	
	Bishop and Folley (1944)		Foss (1942)	Emmens (1941)	Forbes (1943)
	Half life days	Initial daily absorption per cent	Mean daily absorption per cent	Mean daily absorption per cent	9/10 life days
Testosterone (compressed)	43	1.2	1.2	1.2	31
Androstendione (compressed)	50	1.0	—	—	—
Androstendione (cast)	50	1.0	—	—	—
Progesterone (cast)	55	0 9	1.3*	—	88
Testosterone (cast)	57	1.1	—	—	—
Hexœstrol (compressed)	65	1.0	—	—	54
Stilbœstrol (compressed)	68	1.0	—	0.64	51
Testosterone propionate (compressed)	125	0 55	0.85	0.73	61
Testosterone propionate (cast)	125	0.55	—	—	—
Œstradiol dipropionate (compressed)	190	0 26	—	0.25	140
Deoxycorticosterone acetate (cast)	205	0.20	—	—	64
Œstradiol (compressed)	210	0 24	0.38	0 23	180
Œstradiol (cast)	210	0 24	—	—	—

*Compressed pellets.

exactly the same implantation site, and removed those pellets at exactly the same time, we might still get very considerable scatter with those 4 pellets

It does not seem to make any difference in the absorption rate whether you implant pellets into a male or into a female, nor does it seem to make any difference whether the person is showing clinically a deficiency of the particular hormone you implant, or indeed whether the person is perfectly normal. I should like to point out that a lot of this work was definitely experimental. I rather blush now when I see the large number of points upon the œstradiol curve, because in fact one uses œstradiol very little therapeutically by implantation either in women or in men. Some of those œstrogen points were obtained actually in men, youths with acne. However, much of the work was done on women who had complete ovarian agenesis and to whom it was justifiable, it seemed to me, to give really large doses of œstrogen to try and develop their breasts.

We also noticed a tendency to extrusion, which is particularly noticeable for progesterone One can almost go so far as to say that if one implants progesterone subcutaneously, it will certainly come out. Now that we have been using intramuscular implantation of progesterone with a trocar and cannula, we seldom get any extrusion at all. We also found a good deal of extrusion with testosterone propionate; for that reason we abandoned the clinical use of testosterone propionate and use testosterone itself, which does not seem to extrude nearly so much. D.C.A., œstradiol and stilbœstrol have very seldom given us any trouble at all with extrusion.

On a number of occasions we have been able to recover the remains of a pellet, sometimes quite a considerable remainder, although the clinical effect had passed off some time ago. I do not profess to be able to explain this; it is not anything to do with "ghost" formation or with the formation of a capsule, because we found that the pellet forms a capsule quite early, even after a few days.

From the point of view of the clinician, we have a large number of variables, but it is interesting to see that on the whole our rates of absorption do seem to agree fairly well with those obtained by workers with animals. From a

high absorption rate of deoxycorticosterone acetate reported by him.

We gratefully acknowledge our indebtedness to Organon Laboratories Limited for the pellets, which were specially made for this work and supplied to us sterilized ready for implantation.

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Dr. Bishop

This work emphasises very definitely that one cannot predict accurately the daily dose from a pellet implanted into a human being. Apart from the curve of fused testosterone, which unfortunately is the only one we have published so far, and which does fit very well on to the accepted calculated curve, I think it will be obvious to you that many of the other curves show a lot of scatter, which would make it very difficult indeed to predict the daily dose of hormone from a pellet. Unfortunately, however, that has been done to quite a considerable extent, for instance in the treatment of Addison's disease by implantation of D.C.A. Complicated calculations have been made as to daily requirements of individual patients, determined by blood chemistry and so on, and the corresponding number of 100 mg. pellets have been implanted. Some of the early work was based on this, but I do not think that one should go away with the idea that you can just pick out one of these curves and calculate the exact dose of hormone that you have to implant.

The scatter seems to me quite inexplicable. Dr. Folley and I examined carefully some of the points and we found that even when we implanted 4 pellets of the same material into the same patient at exactly the same time and at

FOLLEY: Comparing our results in humans with other results in rats, the rats seem to absorb pellets more quickly. These findings may be related to the respective metabolic rates of man and the rat.

OVERBEEK: I should like to ask Dr. Parkes why thyroxine was absorbed so slowly in the rat when it should be more soluble than the steroid hormones? Perhaps it would be possible to make implantation tablets of the protein hormones?

PARKES: Acid thyroxine is very insoluble. What about the size of implanted tablets?

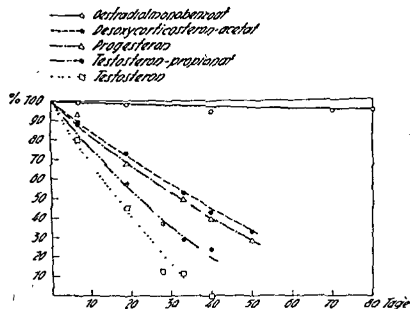


FIG 1. Absorption of 100 mg. pellets of different steroid hormones during shaking in bovine serum at 37°.
Ordinate: Weight of the tablets in per cent.
Abscissa: Days

BISHOP. After using different sizes of pellets, we came to the conclusion that from a clinical point of view probably the 100 mg. pellet is the most convenient unit. If, for example, you wish to implant 700 mg. of D.C.A., we feel it is better to implant it in 7 tablets of 100 mg. rather than in one tablet of 700 mg., because absorption depends upon the surface area of the pellets, and the larger the pellet the smaller the

purely clinical point of view I would say, very roughly, this: 100 mg. of stilbœstrol lasts about 3–5 months, whereas 100 mg. of testosterone lasts roughly from 5–6 months, and 100 mg. of D.C.A. lasts from 8–12 months, and 100 mg. of œstradiol from 12–18 months.

DISCUSSION

PARKES: The figures shown by Dr. Forbes and Dr. Bishop remind me that a 50 mg. tablet of thyroxine implanted into a young rat will keep the animal in perfect health for the rest of its life. The tablet after a year or two will have lost weight at the rate of 1 mg. a year. As far as thyroid hormone is concerned, therefore, one tablet would presumably last a rat for 50 years.

FORBES: I should like to ask Dr. Bishop when extrusion occurred. Was it within a day or two of implantation?

BISHOP: It was very variable. It was not local reaction or anything of that sort. Sometimes the pellet was extruded from a different site from where it had been implanted. Sometimes it occured early; sometimes the pellet had been retained for 42 days or even longer and was then extruded. Testosterone may be extruded 2–3 months after it has been implanted. I don't know what is the reason for this.

BROWNLEE: Has any cellular reaction been noted when extrusion takes place, such as a "cross-over" of white cells? Is there a cytological reaction? I am thinking of the leucocyte change seen after skin sensitivity tests!

BISHOP: I am afraid we haven't looked into that at all.

GROSS: In order to avoid the formation of "ghosts" and of connective tissue around the pellets and to facilitate the determination of absorption time, we tried out a method of shaking the pellets in a tube containing bovine serum, and studied the solution rates of the tablets in the serum at different intervals. The temperature was constantly 37° C. Fig. 1 shows the solution rates for pellets of testosterone propionate, progesterone, deoxycorticosterone acetate and œstradiol shaken in serum We believe that this method of shaking the tablets in serum is suitable for determining roughly "absorption" rates of steroid hormones and has some advantages over the implantation in animals.

GADDUM. Would the time for absorption in serum be roughly the same as on subcutaneous implantation?

GROSS: It would take less than half the time to obtain 50 per cent solution in serum than for absorption in animals.

FOLLEY: Can you get "ghost" formation in serum?

GROSS: We did not observe "ghost" formation *in vitro*. Are there differences in the absorption rates in different species?

ABSORPTION DATA FROM TABLET IMPLANTATION EXPERIMENTS IN RUMINANTS

F. H. MALPRESS

THIS report of work done with Dr. Folley and other collaborators at Shinfield follows appositely the two communications we have just heard, though the data I shall present accrued from work done in 1943 and 1944 which had quite another purpose for its primary aim: our interest was the stimulation of lactation in ruminants and our implantations of œstrogens and other hormones were directed to that end.

Although we have not such extensive data on tablet absorption as Dr. Folley and Dr. Bishop have given you in relation to the implantation in the human, some of our findings may hold considerable interest, and I would like you to notice, first of all, the progesterone absorption figures given in Table I. In all cases the original implant was of a *fused*, one gram tablet; all implants were made subcutaneously in the shoulder region.

Tablet A was removed from a heifer after an implantation period of 22 days, cleaned, dried and weighed, and the daily absorption over this period calculated. It was then reimplanted, as tablet A2, into a steer for 28 days, and afterwards, in a similar way, this same tablet was removed and reimplanted into four goats for varying lengths of time, becoming in order A3, A4, A5 and A6 Thus one tablet was implanted successively into six different animals. You will notice that however many times this tablet was reimplanted the absorption rate remained fairly constant, a result which, for the particular case, conflicts slightly with the general conclusion I intend to make in a moment regarding absorption from these fused tablets.

surface area in relation to weight, and therefore the weaker the dose you actually get. For clinical purposes pellets of 100 mg should be made.

GADDUM: You would not want to use a smaller quantity than that?

BISHOP: There is a tendency in some quarters to use implantation methods for the relief of menopausal symptoms, particularly in women who have had hysterectomy, and in these cases probably 100 mg. is too high a dose and 25 mg. tablets would be suitable. With regard to progesterone, the difficulty of implanting deeply a tablet as large as 100 mg. is very real, and we have had to make long thin cylindrical pellets of 25 mg.

BROWNLEE: Have you tried the effect of using small spheres?

BISHOP: We were approached in one case where a man had had both testicles removed on account of tuberculous epididymitis. He obviously required prolonged, possibly permanent, androgen treatment, because if he did not have androgen treatment he suffered very considerably from hot flushes. It was suggested that we might make an enormous spherical pellet about the size of the testicle and implant it in the scrotum, but clearly this was not a good idea because of the very small daily dose which he would actually get.

FOLLEY: A flat disc would be mechanically at a disadvantage; there would be a tendency for it to crack.

attends implantation, the rate of absorption per unit surface area from these fused tablets remains unaffected by time.

I should like to contrast this general conclusion with the results given in Table II. These figures concern the absorption from one gram, *compressed* tablets of hexœstrol implanted into different animals for varying lengths of time. Here the daily absorption shows a great deal of scatter and it is difficult to escape the conclusion that these values are largely dependent on conditions characteristic of the animal itself, or of the operational technique. The individual response of the animal to a foreign body, the particular site of implantation or, indeed, the precise positioning of the tablet

Table II

DAILY ABSORPTION FROM 1 GM. COMPRESSED HEXŒSTROL TABLETS IMPLANTED IN RUMINANTS

Animal	Duration of implantation (days)	Daily absorption (mg.)
A	42	3 5
A	42	2.1
A	42	2.5
B	46	1.2
C	63	0.9
C	63	0 9
D	69	0.5
D	69	0 6
E	79	1.6
E	79	1.2
F	106	1.4
F	106	1.4
G	124	2.4
G	124	1.5
H	49	1.7
H	49	1.4
H	104	1.8
H	104	2 1

The second tablet, B, broke *in situ* during the first implantation, giving us two half tablets, B2*a* and B2*b*, which were both reimplanted separately in the same animal—a steer; B2*b* was further reimplanted into a goat. Tablets C, D and E, as shown in Table I, were implanted and re-implanted into goats in the same way and call for no particular comment.

Table I

DAILY ABSORPTION FROM FUSED PROGESTERONE TABLETS IMPLANTED IN RUMINANTS

Implant	Animal	Weight of Implant (mg.)	Duration of implantation (days)	Daily absorption (mg)
A1	Heifer	998	22	1.4
A2	Steer	968	28	1.1
A3	Goat	924	66	1.4
A4	Goat	835	56	1.8
A5	Goat	786	80	1.7
A6	Goat	601	83	1.4
B1	Steer	999	42	2.6
B2a	Steer	385	28	0.8
B2b	Steer	504	28	0 8
B3b	Goat	480	107	0 8
C1	Goat	998	80	2.1
C2	Goat	833	83	1.9
C3	Goat	673	107	1.2
D1	Goat	1,000	83	2.5
D2	Goat	795	107	1.6
E1	Goat	999	83	2.3
E2	Goat	807	107	1.8

The general conclusion that would seem to emerge from the collected data on these five tablets is that they probably would conform fairly satisfactorily to the demands of the Folley-Bottomley equation which has just been presented, and show that any decrease in absorption with time was mainly a result of a decrease in the size of the tablets; in other words, if allowance could be made for the surface pitting which

bigger tablets than Dr. Bishop and Dr. Folley were using in their earlier work, and whereas their tablets were completely absorbed, our total absorptions represented a mere 10 per cent. of the whole tablet; in other words Fig. 1 shows, by comparison, just a tiny fractional corner of one of the graphs Dr. Folley has just shown us, and focuses attention on the

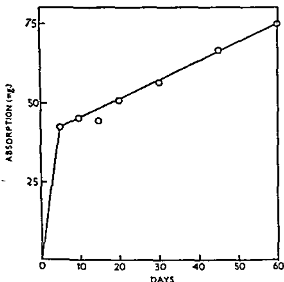


FIG 1. Total absorption from 500 mg compressed hexœstrol tablets implanted simultaneously and in separate sites in a goat for varying periods

possibly atypical initial rates of absorption. I think you will agree that our results with this animal permit us to distinguish two phases of absorption: the first a short-lived phase of very rapid hormone uptake, the second a continuing low rate of absorption which might perhaps be regarded as the "true" rate characteristic of the tablet, provided that we do not overlook the fact that the transitory first phase may be of equal or greater importance physiologically. Perhaps the best explanation that one could put forward to account for this phenomenon, is that in a compressed tablet—and unlike

in that site, might all be influences affecting critically the rate of "ghost" formation in the tablet itself, or the rate of capsule formation around it. The figures show at least a four-fold difference in absorption rates in different animals, whereas they are invariably of the same order for similar tablets implanted into the same animal. This seems to me to be an important point, for since we are so far from assessing likely absorption—to the extent of getting four-fold differences —we are surely playing with a very dubious technique when we implant compressed tablets. Such a variation—say between 0.5 and 2.0 mg. daily—if maintained over even a relatively short time might represent all the difference between a stimulation and an inhibition of the pituitary gland. Certainly the experiments suggest that we have no real control of absorption from compressed tablets within these limits, and it would appear that the no doubt interrelated problems of "ghost" formation, tablet site and capsule formation are facets of the subject which deserve far more attention than has yet been given them.

The constancy of the individual response, contrasted with the unpredictable nature of the response between members

All the implants were made on the same day and placed in well separated sites. The curve shows the total absorption per tablet in mg. plotted against the duration of the implant in days. A five-day implant gave an absorption of about 40 mg.; a 60-day implant an absorption of 75 mg. From this curve it will be apparent that the daily absorption of oestrogen falls sharply after the fifth day, from a value of 8 mg. to a steady value of less than one mg.

It is interesting to find again that when one deals with one animal only, the tablets implanted at different sites should

simultaneous implants showed a scatter in uptake.

There is however a further interest in this particular goat and its absorption rates. The experiment dealt with much

way it was constant in one animal, and different in different animals. Adrenalectomy might have a big effect on the rate of absorption.

BROWNLEE: When taking tablets out of bovines I have observed marked differences in the vascular bed seen around the product. I would think that blood supply might be important.

FOLLEY: In one of our series of humans, 3 or 4 pellets of, I think, œstradiol were implanted successively and in all cases they fell below the threshold This patient seemed to be a slow absorber.

PARKES: With the implantation technique the tablet can be taken out again and the amount absorbed estimated. If absorption seems to be irregular from case to case, or from tablet to tablet, implantation is regarded as a rotten technique. By contrast, you have no idea what happens to an injection, because you can't take it out again to have a look. I think that should be remembered in comparing techniques.

BISHOP: I was interested in the question of taking a tablet out of one animal and putting it into another animal. Did you sterilize the tablet again, or is that talking nonsense?

MALPRESS No, we did not sterilize it; it was very carefully washed, dried and weighed, and then taken away and stored in a desiccator until required again. That seemed to be sufficient for our purposes.

BISHOP Occasionally pellets extrude in human beings, and I have never been able to persuade any commercial firm to resterilize the pellets for me. If we could only have them resterilized and put them back again it wouldn't cost the patient any more, but if we are to have a complete new set of tablets, it becomes quite expensive, and it is a very nice point as to who should pay for the tablets, the man who put in the tablets which came out, or the patient. Is there any objection to resterilizing them?

PARKES. Can't you reconstitute them, in the case of fused tablets?

BISHOP: Nobody will do it.

PARKES Would you clinicians consider it against professional etiquette to put the same tablet back into the same patient ?

BISHOP I think that is all right. I am all for it.

PARKES: Would you put it into another patient?

BISHOP. Yes, I think so

BROWNLEE Stilbœstrol itself is a potent antibacterial substance.

BISHOP So that with stilbœstrol you might be able to replace the pellets

BROWNLEE What I say is also true for testosterone, but not for progesterone, I think.

the fused tablet—it is the rapid removal of a powdery surface layer which ensures the initial accelerated hormone uptake. If this is so the first phase of absorption might represent merely an apparent uptake, since the loss in weight might be due to a mechanical "brushing off" of this powdery surface layer which could remain still in an insoluble, though irrecoverable form. It would seem unlikely that "ghost" or capsule formation could interfere so drastically with absorption values in so short a time, though that may be so, but it should be noted that if the initial high absorption really represents the uptake of the tissues our assessment of the practical advantages of the implantation technique may need some revision; for it is generally thought that when you implant a tablet you will get a steady uptake of your hormone over a long period and that that is one great advantage of the method; but what we possibly got in our implanted goat was a very sudden sharp shock of œstrogen which might well have been big enough to inhibit the pituitary for a considerable time. We may therefore be deluding ourselves if we assume that our implant is giving us a steady stimulatory dose of hormone when we use compressed tablets—we may be getting nothing of the sort.

May I then summarise our interpretation of these absorption figures by saying that they suggest a fundamental difference between absorption from fused progesterone and compressed hexœstrol tablets in the initial stages of an implantation, which we regard as due to the difference in the mechanical structure of the tablets rather than the difference in the hormones of which they are composed; and that further they offer some grounds for postulating a quantitatively characteristic response for the individual animal in contrast to the quantitative variation within the group.

DISCUSSION

BISHOP: We found clinically that in some cases there seems to be evidence of rapid initial or early absorption. One or two eunuchoids

DATA ON PROGESTERONE PHYSIOLOGY AND METABOLISM

T. R. FORBES

THE observation has been made independently two or three times that in the uterus of the mouse there is a difference in the stromal nuclei of the endometrium depending on whether or not the animal was castrate or was pregnant. Dr. Charles Hooker, then at Yale, observed the phenomenon and conceived the idea of combining it with the principle of local application of hormone, more specifically the local application of the hormone by uterine injection, as done by McGinty. Hooker proposed that we might look into the possibility of injecting progesterone solutions, and, if successful, unknown solutions, into a segment of uterus in the castrate mouse as a method of bio-assay. We found that if an animal has been castrated for 16 days the stromal nuclei are always shrunken and fusiform. However, in the presence of a sufficient amount of progesterone the nucleus acquires an enlarged oval outline, a conspicuous nucleolus, and fine, evenly distributed chromatin particles. The injections we first attempted were delivered by means of a tuberculin syringe, graduated into hundredths of ml. However, in the uterus in a castrate mouse after 16 days there is a lot of atrophy. One hundredth of a ml. caused too much distension; the uterine wall was stretched, and we couldn't see the nuclear response. We devised an instrument which could deliver a smaller quantity.

The device we have been using is simply a tuberculin syringe on a metal base, on which is mounted part of a micrometer caliper. By rotating the handle of the micrometer, the bolt

OVERBECK: Can't you just put the pellet in boiling water and sterilize it that way?

BISHOP: Yes, but can you? Can it be done?

FOSS: Before the war when I was doing some experiments along these lines, I used to boil mine in a test tube. That is what I was told to do. It worked very well. No trouble at all.

BISHOP: I think the very first one that we put into a human being was done that way, wasn't it, Dr. Parkes?* That was compressed œstrone and it was boiled.

PARKES: I have just been reminded of the first progesterone tablet that was ever implanted. It was very valuable indeed, and was put successively into a woman, a goat and finally a rabbit.

*Bishop, P. M. F. (1938). *British Medical Journal*, **1**, 939.

After having shown that there is a definite response that can be evoked by progesterone, the next problem was to try to determine the minimal effective dose, the least quantity of progesterone which would give a response. That was done by making up as carefully and as accurately as possible a solution of crystalline progesterone in sesame oil and then injecting successive dilutions of the material until we found the greatest dilution which would give a positive response. We consider the response positive when any nuclei at all in a section show the characteristic effect. If no nuclei show it, the response is negative. Absence of response, of course, does not necessarily indicate total absence of progesterone, but, rather, absence of a sufficient amount of progesterone to give the result. Under our experimental conditions and in this strain of mice, the least amount of progesterone which will produce a positive effect is 0.0002 microgram. This is a very small quantity, but you must also remember that it is a very small piece of tissue that is being treated and that the hormone is applied directly. As nearly as we can tell, the assay is reasonably accurate. I don't think the error is more than 10 per cent.

It was necessary to test the specificity of the response. What we did was to inject a variety of other compounds, ordinarily in oily solution, to see whether they would also evoke the progesterone response. We selected compounds on the basis of whether they may be expected to occur in the plasma, or whether they may be possible precursors or metabolites of progesterone, or are compounds having a close chemical similarity to progesterone.

The assay appears to be quite specific. With *subcutaneous* administration, not only did progesterone give positive results when a sufficient quantity was administered, but so also did deoxycorticosterone acetate and testosterone propionate. The only apparent explanation is that somewhere between the point of subcutaneous administration and the uterus there is a conversion of a part of the material which has been injected.

We had a questionably positive response with an aqueous extract of adrenal cortex in undiluted form. That may represent the action of an unrecognised substance which

individually, of course. There are clips which hold the syringe so that it can be readily removed, washed with ether, and reloaded with another unknown material.

The mice that are used for this assay are the CHI strain of mice, a highly inbred strain developed by Dr. L. C. Strong. I do not know if there is any particular virtue in that particular strain, but I do suspect that it is important to use an inbred strain of mice so that the biological variation can be minimized just as much as possible. We use young adult females, ovariectomised when they are about 70 days old. We wait 16 days before injecting these animals, as it turns out that one may need to wait that long in order for the effects of the endogenous progesterone on the stromal nuclei to disappear.

At the time of the injection the mouse is anæsthetised and a ventral incision is made. One of the uterine horns is delivered. Two ligatures are placed around the uterine horn about 5 mm. apart. The more anterior ligature is tied tightly; the other ligature is left open. A fine needle, No. 27, is inserted through the wall of the uterus into the lumen, making sure that the point of entry is caudal to the caudal ligature. At present we inject 0.0008 ml. of material for assay. With a larger quantity, there is an undesirable degree of distension of the uterus.

The needle is withdrawn, the ligature is closed, and the test fluid is trapped in a segment of uterus. The other uterine horn can then be similarly injected with another material. After both horns have been injected, the abdomen is closed. The animal is killed after two days. The uterus is then removed, fixed in Lavdowsky's solution, sectioned at 6 micra and stained, and then the response is read.

In a cross section of mouse uterus it is easiest to find responding nuclei in the thicker portion of the wall. It is necessary to avoid paying serious attention to nuclei lying close to a uterine gland or close to a blood vessel, as such nuclei give a false positive response. A positive response to progesterone is shown by the fact that some cells, not all of them of course, have an elongated oval nucleus, a conspicuous nucleolus, and fine evenly scattered chromatin particles.

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We had a questionably positive response with an aqueous extract of adrenal cortex in undiluted form. That may represent the action of an unrecognised substance which

duplicates the progesterone effect, or it may well be that progesterone is present, as reported by Beall and Reichstein.

Now, most of these compounds, although not all of them, have some effect on the nuclei. The point was that, as nearly as we could tell, the effect was not identical with that produced by progesterone.

We also tried mixtures of various hormones, that is, of progesterone with œstrogens, progesterone with androgens, and so on. As far as we could tell, the added compounds did not interfere with the response, or enhance it, or diminish it.

In studying bloods, we have worked chiefly with plasma. We have assay values for raw whole blood, raw plasma, ether and acetone extracts of plasma, etc. The figures check out fairly well; that is to say, the values for raw whole blood are lower than for raw plasma; the correction for the haematocrit fits reasonably well. The values for hydrolysed whole blood are higher than those for raw whole blood but not as high as for raw plasma. Hydrolysis, Dr. Hooker and I think, releases the protein-bound material, of which *there is a small proportion, and that is added to the initially* free progesterone. Hydrolysis involves addition of sufficient hydrochloric acid to bring the pH down to about 1, and then exposure to the temperature of boiling water for 6 hours, the obvious purpose being to break down the proteins thoroughly, and thereby to release any progesterone which might be protein-bound.

Actually, we have no proof as such that progesterone is really bound to protein, but it seems likely that that is the case.

We were interested in the bound progesterone, and the question came up as to whether it had any physiological activity. We made some progesterone pellets. In one group of ovariectomized mice the pellets were implanted subcutaneously; in another group, the pellets were implanted intra-splenically. Then, at intervals after implantation, the mice were bled by cardiac puncture, and the plasma from the blood samples was subjected to assay. We found no splenic adhesions at autopsy. Our purpose was to try to direct all the progesterone through the portal circulation. If there had been splenic adhesions, some of the hormone could have

escaped into the general systemic circulation before going through the liver. In the case of the subcutaneous pellets, there was a steady rise in the progesterone level. The free

the subcutaneous pellets showed positive responses as soon as the level of free progesterone got up to about 1 microgram per ml. In the case of mice with intrasplenic pellets the levels of *bound* progesterone rose to about 2 micrograms per ml. and apparently levelled off, but the levels of free progesterone never got higher than 0.6 or 0.7 microgram per ml., and in no case did the uteri of these animals show positive responses. We interpreted this to mean that, in the first place, the bound progesterone was physiologically inactive, since we had a positive response with a high level of free and a high level of bound progesterone, and here we had a negative response with a low level of free and a high level of bound progesterone. Secondly, we think this indicates that one of the methods of hepatic inactivation is by binding to proteins. Again it is not proven that the inactivation, if that is the right word, took place in the liver. It is possible that inactivation occurred in the spleen or even in the portal vein, but that seems less likely in view of collateral evidence.

In connection with this process of inactivation, or degradation, or whatever it is that happens to hormone, we decided also to study progesterone levels in renal blood. That was done by assaying blood samples either from pseudo-pregnant rabbits or from monkeys in the luteal phase of the menstrual cycle. We chose these animals in the hope that they would have appreciable amounts of progesterone in circulation. Under anæsthesia, a sample was drawn first from the renal vein and then from the renal artery.

appreciably higher than the free progesterone level in the renal vein. There was a range from 16 per cent reduction to 94 per cent reduction in the rabbit, and a similar range in the monkey. This reduction occurred whether there was a high level of progesterone in the renal artery or a relatively low level. The kidney seems to be doing something to the free progesterone.

It is possible to make an interesting rough calculation. If we assume that we have a monkey with 6 micrograms progesterone per ml. plasma and that the monkey has a total of 300 ml. of plasma, then there would be in circulation at a given moment 1,800 µg., or 1.8 mg., of the hormone. But if we assume that as a result of renal passage that value is reduced by 67 per cent (which is somewhere around the [illegible]

one looks up figures on the rate of renal flow it appears that the equivalent of all the blood in the body may flow through one or the other of the kidneys in perhaps 4 minutes, in which case the rate of removal or inactivation of progesterone would be as much as 0.3 mg. per minute. This suggests, further, that if a given systemic level is to be maintained over a given period of time, then at least this much progesterone must be added to the circulation, or the level would drop. I say "at least this much" because, at least in some forms, hepatic inactivation is also in operation, and perhaps additional mechanisms not yet clearly recognized. Inactivation at the rate of 0.3 mg. of progesterone per minute is very surprising, particularly in terms of the body's economy. It is difficult to think of the body producing progesterone as fast as that and then discarding it. This suggests the further possibility that there may be a reconversion of the metabolite back to progesterone. I know of no evidence at all for such an assumption, but it seems just possible that after pregnanediol, for example, is removed, it may be reconverted elsewhere to progesterone, a process comparable to the repeated utilization of bile acids that has been demonstrated in the digestion of fats.

We did another experiment, trying to get some idea of plasma progesterone levels following subcutaneous administration of the hormone to castrate mice. We have only done this with a relatively small number of animals, and again I would like to present this with some reservations. We made daily injections of 0.5 mg. of progesterone in oil subcutaneously in each of a series of mice, and then, following the last injection, bled the mice at intervals and assayed the plasma. The first animal was bled after 6 daily injections, that is, after receiving a total of 3 mg, and had a level of free progesterone of about 2 µg. per ml. This was 24 hours after the 6th injection. The next animal was bled 3 hours after the 7th injection, had received a total of 3.5 mg., and had a level of 5 µg. per ml The level was at 5 µg per ml. at six, nine, and twelve hours after the last injection, but in the animal bled 24 hours after the last injection, the level was down to 1.2 µg. per ml. It would certainly appear, on the basis of this very small experimental group, that a single daily subcutaneous injection of progesterone in oil in mice is not sufficient to maintain a continuously high level of plasma progesterone.

I would like to tell you of some studies on the human menstrual cycle. The donors were four apparently healthy young women who were willing to give 1 ml. blood samples. They were instructed to record their waking temperature, the so called "basal temperature," taken orally each morning. As far as the temperature curves go, we found the same sort of thing that has been reported repeatedly in the literature, that is, that the waking temperature is relatively low during the follicular phase, and is elevated during the luteal phase. It is commonly believed, very likely correctly, that the sudden elevation at mid-cycle coincides with the time of ovulation. Certainly the elevation coincides with or just follows the appearance of progesterone. An interesting thing was that free progesterone was present at a concentration of about a microgram per ml. on the first day of menstruation, and was still present two days later, then fell off, and presumably disappeared. We found that the curve for the progesterone level pretty well coincided with the curve for the elevation of temperature.

In one case the temperature record was very confusing because it fluctuated. Then we got the results of the assays, and we could not find any progesterone. Apparently she was running an anovulatory cycle, which may have accounted for the atypical temperature record. The succeeding cycle appeared to be normal. There was a very pronounced temperature dip, then a rise about the time of the appearance of progesterone. Here again the hormone was still present after menstruation had begun.

One donor experienced Mittelschmerz during the cycles we were following. On the 17th day her temperature record was low at 7 a.m.; her blood sample was drawn about 11.30 a.m., and subsequently showed the presence of progesterone; between 11 and 11.30 that night she experienced Mittelschmerz. So progesterone was present in the plasma at least 12 hours before Mittelschmerz occurred. She had a typical cycle with a very strong elevation of temperature, then the temperature dropped as menstruation began. In this case the progesterone, a relatively small quantity, persisted throughout the menstrual period. We took samples subsequently at what we thought might be the time of a later ovulation, and Mittelschmerz occurred again. (This is the only case I have observed where progesterone appeared during the follicular phase of the human cycle, although we have done a little work with levels in the monkey during the menstrual cycle, and one of the monkeys has shown progesterone during the follicular phase.) Progesterone was present in a sample taken about 10 a.m; progesterone was absent in the sample taken the next day at about 10.30 a.m.; and Mittelschmerz occurred at noon, so that the hormone was present approximately 24 hours before Mittelschmerz, was absent one and a half hours before Mittelschmerz, and then reappeared subsequently. Now it is probably not proper to attempt to pool these data, but if one did, it would appear that progesterone is present well in advance of Mittelschmerz, is absent during the time of the phenomenon, and then reappears afterwards.

It is clear that in a good many of the cases progesterone is present during menstruation, although it is true that there [illegible] been found a depression of the level of the hormone prior

to the onset of menstruation. In all cases there is good correlation between the appearance of progesterone and the elevation of the basal temperature. This phenomenon of progesterone appearing at or before the time of ovulation has been postulated several times by other workers on the basis of indirect evidence. It seems to occur here.

I have been checking on it with pseudo-pregnant rabbits. What we have done with rabbits is to take a control sample of blood from an ear vein, then to inject gonadotrophin, and then at intervals of one or two hours thereafter, to take successive blood samples from the ear vein. In the control sample we observed little or no progesterone—the quantity present was less than 0.2 or 0.3 μg. Then gonadotrophin was injected to induce ovulation. Two hours later progesterone appeared in the plasma, at a level of about 1 μg. per ml. It was not present one hour before. The significance of this is that ovulation occurs about 9½ or 9¾ hours after mating, after a simulation of mating, or after injection of gonadotrophin, so that progesterone was present perhaps seven hours before the assumed time of ovulation. Ovulation was not observed in these rabbits, but subsequent laparotomy or autopsy did show the presence of ruptured follicles. So, apparently, in the rabbit progesterone is released before rupture of the follicle and before organization of the corpus luteum. Where it comes from is anybody's guess.

It seems to me not entirely impossible that individual, scattered luteal cells may be present before the corpus luteum as such is organized. That is something that can be worked out histologically. Professor Hammond implies in his work on the rabbit that this situation exists. There are a couple of other supporting points: a report by Gillman and van der Horst on the elephant shrew, a curious South African rodent, in which, it is said, the corpus luteum forms before ovulation, and actually is responsible for ovulation. And Seaborn, in a paper written in 1925 on the œstrous cycle in the mare, has described the presence of luteal cells in the follicular wall

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DISCUSSION

MALPRESS: I would like to suggest that in the technique of paper chromatography you might well find a chemical method which you could successfully adapt to your problem; it is well suited to work involving such small quantities of material Have you done any chromatographic separations?

FORBES No. I hope that can be worked out. There is some work, I think, being done along this line.

There is one other compound I would like to mention, a so-called X-compound, which Pearlman isolated from ox bile and which we found does duplicate the progesterone effect. It isn't progesterone because it has a higher melting point. Pearlman thinks it may be an isomer of progesterone.

PARKES· Have you any explanation for the occurrence of progesterone in the blood of cockerels?

certainly not orthodox, but it has been shown that œstrogens are present in stallion's urine, and pregnanediol in the urine of bulls.

This field is, at the moment, extremely confusing. There are some people who are beginning to emphasize the importance of œstrogens as stimulating the placenta to form progesterone in cases of abnormal pregnancy. The extremely large volume of work which has come from

the Smiths* is very striking, and rather suggests that the administration of œstrogens is a cure for any kind of disorder of pregnancy from habitual abortion to threatened abortion, toxæmias, pre-eclampsia, and eclampsia.

FORBES We have always found low levels of bound progesterone in all physiological human states.

BISHOP: Have you any information about progesterone in human pregnancy?

FORBES: We are working on that now, taking samples once a week and doing assays. I don't know how long it will be before we get some conception of what the typical curve might be. We did get 6 or 7 samples from a case of threatened abortion, the first during the 9th week of pregnancy and the last one during the 10th or 11th week. Only one sample contained progesterone

BISHOP: Even with large doses of stilbœstrol?

FORBES: Yes. The Smiths believe, if I understand the matter

Smiths§ claim that it's no good giving œstradiol, you must give stilbœstrol, and that that is the substance that stimulates the placenta.

KLEIN: Which androgens did you use in your tests for specificity?

FORBES Testosterone, androsterone, and methyltestosterone

KLEIN I asked because Parkes and I showed that methyltestosterone has a progestational effect on the endometrium when injected into female rabbits.

FORBES We tried it, and the response was negative. The test has nothing to do with whether a substance has a physiological effect

*Smith, O. W., Smith, G. van S, and Hurwitz, D (1946). *Amer. J. Obstet. Gynec*, 51, 411.

†Davis, M. E, and Fugo, N. W (1947, 1948). *Proc. Soc. exp. Biol, N.Y*, 65, 283, 66, 39, 69, 436.

‡Sommerville, I. F, Marrian, G F, and Clayton, B. E. (1949) *Lancet*, 1, 680.

§Smith, O. W (1948). *Amer J. Obstet. Gynec.*, 56, 821

FORBES: I would like to call your attention to a paper by Hertz and [illegible]

the œstrogen per ml. of serum. The level had dropped away down within 3 hours, and within 8 hours it had disappeared.

MACAULAY: In your experiments with subcutaneous implantation of progesterone pellets, how much progesterone did you implant?

FORBES: About 3–4 mg.

A student who has been doing a thesis under Dr. Hooker and myself has been able to maintain pregnancy in an ovariectomized mouse by administering progesterone, provided that he starts progesterone administration as soon as he does the ovariectomy. If the ovariectomy is done on the 6th day of pregnancy, he has to give 8 mg per day (2 injections of 4 mg each), and the amount required gradually diminishes if ovariectomy is done later in pregnancy.

MACAULAY: You found that in the subcutaneous implantation method there was a stromal nuclear reaction when the level of free progesterone reached 1 microgram per ml., and that where the level of free progesterone was less than 1 microgram there was no response. Doesn't this indicate that the threshold blood level would be about 1 microgram per ml.?

FORBES: Yes.

DEANESLY: Dr Forbes has shown that progesterone must be produced by other parts of the organism than the corpus luteum. That has, I think, been shown in the pregnant mare where the luteinized follicles arising in the ovary contained progestogen.

PARKES: How many man-hours does it take to do one of these estimations? It is quite a laborious method?

[illegible] 10 or 15 mins.

started on Tuesday, the slides are ready by Wednesday, and we inject again on Thursday.

PARKES. I suppose the number of mice for one determination depends on how lucky you are?

FORBES: Yes, how lucky you are at guessing what the level is going to be.

PARKES: How many mice do you have on each experiment?

FORBES: For the first menstrual cycle, there were 20 samples to be assayed and we used 89 horns—that's 44½ mice. But there the levels were not too hard to predict. When we got to pseudo-pregnancy of the rabbit, where the levels were fluctuating greatly, it was very much more difficult.

OVERBEEK: Is it absolutely essential to use mice?

FORBES: We haven't tried it with rats, but I think it would be possible. There is an abstract in the Anatomical Record (1949. *Anat.*

[illegible]

œstrogen, 3 or 4 ten-millionths of a microgram. They made a very rough calculation, I was told that it was about 200 molecules per cell. I think that's not too unreasonable when one considers that progesterone is a relatively inactive compound.

PARKES: What about the strain of mice?

FORBES: It is important. A man in our laboratory who had a surplus of another strain decided to try our test with that strain. Whereas our minimal effective dose was approximately 0 0002 microgram, his was approximately 0 0008.

PARKES: If the strain were a little sensitive, you wouldn't mind? You've got some margin

MACAULAY: You don't really have very much margin do you? If the blood level of progesterone necessary to produce a stromal nuclear

[illegible]

do you?

[illegible]

17-KETOSTEROID EXCRETION AND MODES OF ADMINISTERING TESTOSTERONE PREPARATIONS

CHRISTIAN HAMBURGER

WHEN treating patients with large quantities of testosterone it is desirable to carry out the treatment in as economical a manner as possible, that is to obtain the best utilization of the hormone. The question then arises, whether treatment with oily solutions, suspensions of crystals, emulsions, or tablet implantations is to be preferred, and what effect the difference in the mode of administration has on the dose and frequency of administration. A mere evaluation of the clinical effect of *the treatment is not likely to serve as a guide, and we have* therefore investigated what information could be obtained by assaying the urines for their content of 17-ketosteroids.

It has repeatedly been demonstrated that the administration of testosterone preparations to human beings is accompanied by an increased excretion of androgenic substances and 17-ketosteroids. The amount of testosterone propionate recovered as 17-ketosteroids after intramuscular injections of oily solutions varies individually from a few per cent to more *than 70 per cent. On an average 40 per cent of injected* testosterone propionate is excreted as 17-ketosteroids. When calculating the amount of testosterone propionate degraded to 17-ketosteroids, the difference in the molecular weights of testosterone propionate and of the 17-ketosteroids (e.g. [illegible] must be taken into account. [illegible] amounts

We have employed the following technique for the assay of 17-ketosteroids : 1/50 part of a 24-hour urine is hydrolyzed with sulphuric acid and after chilling extracted once by vigorous shaking with ether in a separating funnel. The ether extract is purified in the usual way, and the rest taken up in 0.8 ml. of absolute alcohol. The colorimetric reaction is

then performed in duplicate according to the method of Callow. The colour is measured in a Coleman Spectrophotometer, and the values obtained are corrected for non-specific urinary chromogens.

It is a well-known fact that the endogenous production of sex hormones is depressed during administration of œstrogenic or androgenic substances, and that this depression is due to a decreased production of hormones from the anterior lobe of the pituitary gland. This hypophyseal inhibition creates some difficulties in the calculation of the amount of injected testosterone preparations excreted as 17-ketosteroids, because it is not possible to know with certainty on which day of the treatment the diminution of the endogenous 17-ketosteroid production begins, and how much it amounts to. The inhibition probably varies in different individuals and with the intensity of the treatment. One example of the inhibition of the endogenous 17-ketosteroid production

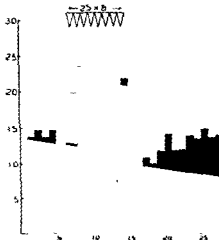


FIG 1 Daily excretion of 17-ketosteroids before, during and after the administration of 25 mg. testosterone propionate daily for eight days in oily solution.

In Figs 1–6 the white wedge-shaped triangles indicate the injection of an oily solution, dotted triangles the injection of an emulsion, and black triangles the injection of a crystalline suspension The figures above the triangles show the amount of substance in milligrams

is shown in Figure 1; *the experimental person was myself.* The average daily excretion for a period of 6 days before the first injection was about 14 mg. per 24 hours. Daily injections of 25 mg. testosterone propionate in oily solution (Perandren) were given intramuscularly for 8 days. The excretion rose gradually and reached a maximum on the 3rd day (about 28 mg.), and this value was maintained for 3 days. In spite of continued injections the excretion then fell to about 22 mg. After discontinuation of the treatment subnormal values were observed for one week, the lowest values being 9 to 10 mg. per 24-hour urine. We can now make some calculations, based on the ascending part of the excretion curve, assuming that one injection of 25 mg. Perandren gives rise to an extra excretion of 6 mg. 17-ketosteroids in the first 24 hours, 4 in the next, then 3 and nil on the fourth day (see Table I).

After the fifth injection the values obtained are on the average 5 mg. too low. This seems to indicate that during the administration of 25 mg. testosterone propionate intramuscularly daily an inhibition of the hypophyseal function commenced in this case after 5 injections, and this caused a decrease in the endogenous 17-ketosteroid production, amounting to about 5 mg. per day, that is about 30 per cent of the daily output.

In some instances a single intramuscular injection of testosterone propionate in oily solution is sufficient to produce a hypophyseal inhibition. Figure 2 shows the 17-ketosteroid excretion after a single intramuscular injection of 50 mg. Perandren in oily solution and later 150 mg. After both injections high excretion was followed by values 2 to 3 mg. below the average pre-treatment value. You will [illegible] tion after single injections [illegible] figures.

[illegible] us 17-ketosteroid production makes it impossible to calculate exactly the percentage of testosterone propionate excreted as 17-ketosteroids. We have, therefore, calculated the additional excretion partly [illegible]

[illegible]

Table I

		*	*	*	*	*	*	*	*					
Endogenous . . .	14	14	14	14	14	14	14	14	14	14	14	14	14	14
Additional . . .		6	4	3	0									
			6	4	3	0								
				6	4	3	0							
					6	4	3	0						
						6	4	3	0					
							6	4	3	0				
								6	4	3	0			
									6	4	3	0		
Calculated . . .	14	20	24	27	27	27	27	27	27	21	17	14	14	14
Found . . .	13	20	23	28	28	27	23	21	22	15	10	11	10	12
DIFFERENCE . .	−1	0	−1	+1	+1	0	−4	−6	−5	−6	−7	−3	−4	−2

The days of injections are marked with an asterisk (*).

present during the whole period. The true value must then be found somewhere between these limits.

The following figures show a comparison of the 17-ketosteroids excretion pattern during administration of Perandren in oily solutions and in crystal suspensions given as single intramuscular injections. The patients were women with

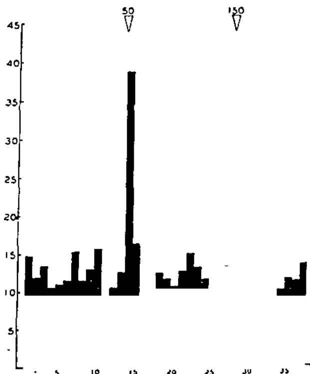


FIG. 2. The effects of two injections of testosterone propionate in oily solution on the excretion of 17-ketosteroids.

uterine cervical carcinoma. Figure 3 demonstrates the high 17-ketosteroid excretion in the first 24-hour urine after 200 mg. Perandren in oil (an increase from 4 to 47 mg.). The increased excretion lasted for 5 days; the hypophyseal inhibition was insignificant, and from 48 to 51 per cent of the testosterone propionate injected was excreted as 17-ketosteroids. Later on she received 200 mg. Perandren as a

crystal suspension. The 17-ketosteroid excretion is lower than after oily solution, but lasts for a longer period of time (about 14 days), and the highest excretion occurred on the fifth day. The total amount of testosterone propionate recovered as 17-ketosteroids was almost the same as after the oil injection.

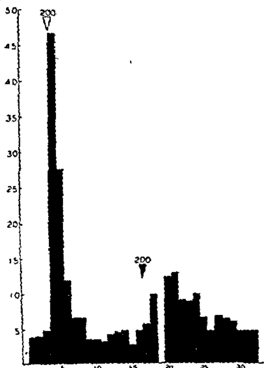


FIG. 3 The effect of testosterone propionate in oily solution and as a crystalline suspension on the 17-ketosteroid excretion.

Figure 4 is from another patient treated in exactly the same manner, and the results were identical with those from the former case.

In the case shown in Fig. 5 the amount of Perandren crystals injected was 500 mg. Increased 17-ketosteroid values were found for about 14 days, with the maximal

excretion on the 3rd day, while the period of increased excretion lasted for 4 days after the oily solution and the highest values occurred on the first and second day.

From these and several other experiments it is obvious that the absorption of testosterone propionate from oily solutions is completed within 3 to 5 days, whereas the absorption of the testosterone propionate crystals lasts on an average for 14 days. The utilization of the hormone as calculated from the total additional excretion is the same.

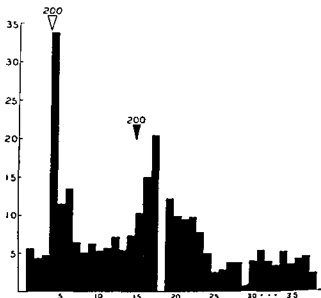


FIG. 4. Another patient treated in the same way as the case depicted in Fig 3

Another patient (Fig. 6) (a woman with carcinoma of the mammary gland) received at first two injections of Perandren in oil, then a single injection of 100 mg. crystals, and some time afterwards 100 mg. crystals every other day for 9 days. The highest excretion of 17-ketosteroids occurred on the day after the last injection, and 14 days later the value was lower than before the commencement of the injections. This case shows that the duration of the absorption period is

independent of the amount of crystals injected and probably is determined by the average size of the crystals.

We have furthermore made some experiments with subcutaneous implantation of Perandren pellets. A woman with carcinoma of the mammary gland (Fig. 7) at first received 50 mg. Perandren intramuscularly in oily solution;

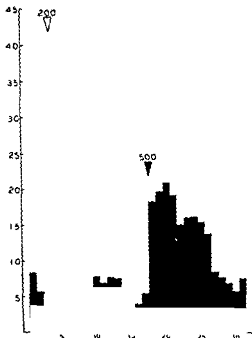


FIG. 5 The 17-ketosteroid excretion after 200 mg. testosterone propionate in oily solution and 500 mg. as crystalline suspension.

some days later 7 pellets of 100 mg. (altogether 700 mg. testosterone propionate) were implanted subcutaneously. 28 days after the implantation, the 17-ketosteroid content was at the pre-treatment level, and on the 42nd day it was sub-normal. It is reasonable to assume that some absorption has taken place beyond the 28th day, but that the daily amount absorbed was so insignificant that it merely compensated for the decrease in the endogenous 17-ketosteroid

excretion on the 3rd day, while the period of increased excretion lasted for 4 days after the oily solution and the highest values occurred on the first and second day.

From these and several other experiments it is obvious that the absorption of testosterone propionate from oily solutions is completed within 3 to 5 days, whereas the absorption of the testosterone propionate crystals lasts on an average for 14 days. The utilization of the hormone as calculated from the total additional excretion is the same.

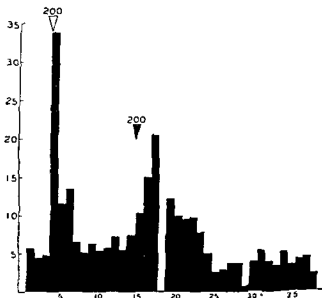
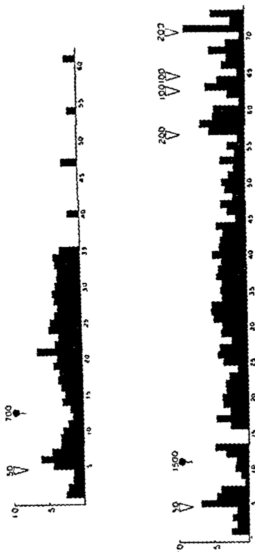


FIG. 4. Another patient treated in the same way as the case depicted in Fig. 3.

Another patient (Fig. 6) (a woman with carcinoma of the mammary gland) received at first two injections of Perandren in oil, then a single injection of 100 mg. crystals, and some time afterwards 100 mg. crystals every other day for 9 days. The highest excretion of 17-ketosteroids occurred on the day after the last injection, and 14 days later the value was lower than before the commencement of the injections. This case shows that the duration of the absorption period is



Figs 7 and 8 17-ketosteroid excretion after subcutaneous implantation of testosterone propionate tablets. The black circle indicates the time of implantation, the figure above it the quantity of the substance in milligrams

Figure 8 shows the result of implantation of 15 tablets (1,500 mg.). A moderately increased level was maintained for 5 weeks, and on the 46th day the 17-ketosteroid content

production. Calculations of the percentage of propionate recovered as 17-ketosteroids gave the the utilization of the tablets was rather incomple pared with the utilization of testosterone propion solution.

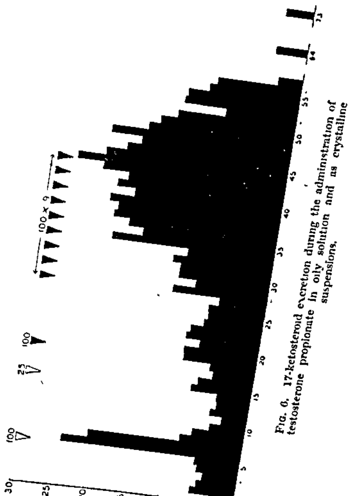


FIG. 6. 17-ketosteroid excretion during the administration of testosterone propionate in oily solution and as crystalline suspensions.

on the fourth day, the pre-treatment value was reached; it was followed by a hypophyseal inhibition and a gradual increase to the normal values. One of my assistants at first received 100 mg. emulsion and showed a moderate increase for 4 days. Later on 90 mg. Perandren in oily solution was given, and increased values were found for 3 days. The two other experiments for which I have no illustrations gave essentially the same results. In all instances the maximal excretion occurred on the first day. Apparently a large amount of the hormone is absorbed from the small globules of the emulsion before the crystallization has taken place, and probably the crystals formed are smaller than those present in the Perandren crystal ampoules. The absorption seems to be slightly slower than after oily solutions, but far more rapid than after injections of crystals.

We have also studied the absorption of testosterone through intact human skin (Fig. 10). In the course of 5 days

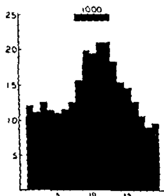


FIG 10. 17-ketosteroid excretion before, during and after percutaneous application of altogether 1,000 mg of testosterone.

I rubbed an alcoholic solution of free testosterone (not the propionate) into my skin, in the morning and in the evening, altogether 1,000 mg. The 17-ketosteroids increased to about [illegible] mg. and afterwards fell [illegible]

The total additional excretion was calculated to amount to

was as low as 1 mg. The absorption period lasted for from 35 to 46 days, and the utilization amounted to about 50 per cent.

These experiments seem to indicate that the life of the tablets is somewhere between 4 and 7 weeks.

We have made 4 experiments with the testosterone propionate emulsions, which were kindly put at our disposal by

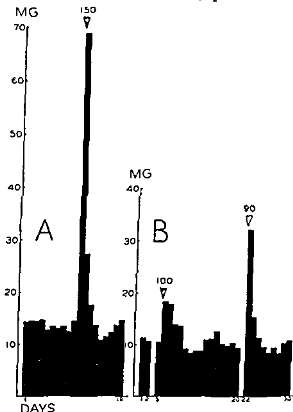


FIG. 9. Injections of testosterone propionate and the urinary excretion of 17-ketosteroids. *Ordinates*. 17-ketosteroids in mg. per 24 hours. *Abscissæ*: days of observation period.

Organon. Figure 9 contains data from two of these experiments. At first I injected myself with 8 ampoules of 50 mg. each, and the 17-ketosteroid excretion rose from an average of 13.7 mg. to 69 mg. in the course of the first day. Already

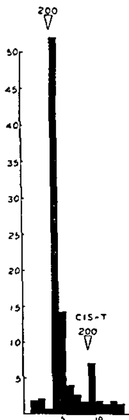


FIG. 11. 17-ketosteroid excretion after intra-muscular injection of 200 mg. testosterone propionate and, later on, of 200 mg of *cis*-testosterone.

after injection of crystalline suspensions, and for about 4–7 weeks after subcutaneous implantation of tablets.

(2) The ultimate absorption of testosterone propionate from intramuscular [illegible]
of crystals seem to
order : implantatic
percutaneous administration.

as 17-ketosteroids, the percutaneous application seems to be only 1/10 as efficient as the intramuscular injection.

I have also tried testosterone propionate percutaneously, but the utilization was found to be still poorer than of the free hormone.

The oral administration of testosterone propionate was examined in another experiment. I swallowed the contents of two 50-mg. Perandren crystal ampoules with water on an empty stomach. By two hours afterwards an increased 17-ketosteroid excretion was found. In the urine from the next day the excretion was only 1.6 mg. above the average, and calculations gave the result that 20 per cent of the testosterone propionate had been utilized. This finding, however, does not mean that we can expect the same clinical effect by giving 5 times as much testosterone propionate orally as intramuscularly. The hormone absorbed from the intestinal canal must pass through the liver before reaching the general blood circulation, and the amount of testosterone propionate escaping the hepatic inactivation might rapidly be degraded to biologically less active compounds, such as androsterone or dehydrosandrosterone or to biologically inert substances such as ætiocholanolone. In that case the clinically effective dose of hormone must necessarily be higher than that expected from the rate of absorption, as calculated on the basis of the 17-ketosteroid excretion.

Finally I should like to show a diagram (Fig. 11) of the 17-ketosteroid excretion in a woman treated at first with testosterone propionate and afterwards with cis-testosterone. This substance was eliminated as 17-ketosteroids only to a very slight extent (2–3 per cent.)

Our results may be summarized briefly in this way:

(1) An increased 17-ketosteroid excretion was found for 1 day after oral administration of testosterone propionate; for 3 days after percutaneous application of testosterone in alcoholic solution; for 3–5 days after intramuscular injection of oily solutions of testosterone propionate; for a slightly longer period after injection of emulsions; for about 14 days

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DISCUSSION

OVERBEEK: I would like to bring into this discussion the conclusions which can be drawn from excretion experiments in general, quite [illegible]

(3) A fairly uniform daily absorption could be obtained when the intramuscular injections of oily solutions were given daily, the injections of crystal suspensions once a week, and the implantation of tablets once a month.

(4) In both sexes the administration of testosterone propionate decreases the endogenous 17-ketosteroid production. As the 17-ketosteroids in female urine probably originates solely from the adrenal cortex, there seems to be an inhibition of the secretion of adrenocorticotrophic hormone as well as of the gonadotrophic hormones.

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output might occur several days earlier than was indicated directly, and that perhaps more of the exogenous androgen was being catabolized than was indicated, since the 17-ketosteroid excretion represents the total metabolic output of exogenous and endogenous androgens. In other words, there might have been a very different impression of the production of the endogenous hormone.

HAMBURGER: Yes.

BROWNLEE: As a pharmacologist, I wonder whether it's fair to put all the emphasis on 17-ketosteroids. It is known that in bovines considerable quantities are excreted in the bile I suppose it is fair to think of the bile as a vehicle for the excretion of steroid hormones, together with the products of their catabolism, and I wonder if there might be some explanation here for the different views about elimination.

BISHOP: I was interested in the apparently very rapid disappearance of testosterone propionate from the pellets It has been our experience that, as far as clinical effects are concerned, pellets of testosterone, when implanted into eunuchoids, control their symptoms for as long as 5–6 months. It isn't that they just feel better for that period of time, but at the end of 5–6 months they begin to get their hot flushes again These are male castrates who had reached adult life before the testicles were removed They are people who are particularly subject to hot flushes Their hot flushes don't come back for at least 4–5 months, and we can usually keep them quite comfortable on two implantations a year. That is my experience. I don't know if Dr. Foss will agree with me?

FOSS: Yes, I agree entirely I think 5–6 months is the average figures

GADDUM: Is it the same material, testosterone propionate?

BISHOP. No, testosterone However it would appear from the figure. Dr Folley showed yesterday that probably testosterone propionate would last even longer. The reason we gave up using testosterone propionate in pellets was that it tended to extrude more frequently than testosterone. It is much more powerful too. In Dr Hamburger's experiments he took himself as the test object, and he is a normal man. If you implant this stuff in people who are definitely lacking androgens, I wonder whether you might get a different sort of metabolism of the exogenous androgens administered.

OVERBEEK It should be done on a hypophysectomised man

BISHOP. Yes, we haven't very many hypophysectomised men. We have done implantations of testosterone in one or two cases of severe Simmond's disease, and our impression again has been that the effect lasts for about 5–6 months.

GADDUM. Is it possible that when Dr. Hamburger attains the pretreatment level, he has just reached a state of balance between depressed natural ketosteroids and slow absorption? Perhaps this drop is an overshoot, and for some time afterwards the material is still being

I think it might lead him to draw the wrong conclusions if he based himself on the excretion. For instance, in this comparison between oily solutions and emulsions, the excretion is almost the same, but both Dr. Gross's and my own experiments show that there is quite a big difference in the effect. I must make it clear that you did not say anything of the kind, but it might be misleading to a clinician.

HAMBURGER. If we compare the oily injections and the emulsions, we find in both cases that after 3–4 days the absorption of the hormone is so low that the rate of absorption is insufficient to compensate for the

must conclude from the experiments that a very large amount of the emulsion is absorbed within the first 24 hours, and that almost the same amount is absorbed from the emulsion as from oily solutions. Our experiments were carried out with testosterone propionate only; we don't know anything about the other hormones. We saw several examples yesterday which indicated that testosterone propionate seems to be more regularly and more rapidly absorbed, when given in tablets or crystals or any other form of administration, than are the œstrogenic hormones, deoxycorticosterone acetate, and progesterone

OVERBEEK Yes, but only 40 or 50 per cent is excreted. What happens to the other 50 per cent?

HAMBURGER· *Probably they are degraded to products other than* 17-ketosteroids.

OVERBEEK: It is possible that what happens to this 50 per cent is quite different in the oily solution from in the emulsion, and that might be the explanation for the difference in the effect of the preparations I quite agree that these results prove that a lot must be absorbed from the emulsions fairly rapidly. I would like to know what happens to the rest because there is such a lasting effect. It makes all the difference whether you inject an emulsion or an oily solution. You can show it with the capon test, for instance.

HAMBURGER: Do you think the testosterone propionate could be degraded, catabolized in another way?

OVERBEEK· That might be the explanation, but it only shows that it is very difficult to compare the absorption, the excretion and the activity, because the excretion is the same but there is a difference in the activity.

HAMBURGER: Don't you think that almost all the testosterone propionate is absorbed from an oily solution within the first 4 days?

GADDUM: Some of these differences may be due to the fact that *Dr. Hamburger is studying man and Dr. Overbeek the capon.*

FORBES· I was very impressed by the drop in the 17-ketosteroid excretion which you interpreted as due to depression of gonadotrophic hormone. It seems possible to me that the drop in gonadotrophin

ADMINISTRATION OF SEX HORMONES AND SEXUAL BEHAVIOUR

M. KLEIN

After the highly skilful demonstration of techniques which we had yesterday and the even more scholarly discussions that followed, I am afraid that what I am going to say to-day will seem rather plain and perhaps even naïve to some of you, because I should like to speak about sexual behaviour in connection with administration of steroid hormones.

I feel I should refrain from discussing behaviour itself and the notion of behaviour; otherwise we shall spend about 5 or 10 hours or more in philosophical considerations about behaviourism. What we call behaviour is the overt reaction of the animal in definite circumstances. We take the whole body as the reactive organ, that is, we just look at the animal to see how the animal behaves in definite conditions. The study of behaviour has grown tremendously during the last 30 years since the start of the behaviourist school in the United States. I have just come back from the States, where I was impressed with the way in which studies of behaviour on the whole, and especially of sex behaviour, have grown there. I was very surprised to see that those studies are carried out very often by psychologists and not by biologists, and I personally feel that behaviour should be included in biological studies as well as the study of any other test.

As far as œstrogens are concerned, I think it is important to remember that œstrogen means generator of œstrus, that is to say, generator of a quite definite behaviour. Very often this notion is forgotten, and a substance is called an œstrogen if it transforms the epithelium, which gives a definite cytological reaction. This may be a very nice reaction for histologists to work out, but it is generally forgotten that œstrogen should give œstrus as a whole, that is to say, the behaviour of the female resulting eventually in

absorbed but the pituitary compensates by depressing the endogenous production of 17-ketosteroids. Might that be so?

HAMBURGER: Yes.

FOLLEY. I think Dr. Brownlee's point is very important in the case of ruminants, since there is reason to believe that in certain ruminants the bile is a particularly important route for the excretion of ketosteroids American workers have shown that cow manure is a very potent source of androgens. In experiments on goats which Professor Marrian did

GADDUM: Have you any people with biliary fistulæ?

BISHOP: Yes, after a gall bladder operation one could possibly collect the bile. In certain cases it would be interesting.

in the œstrous cycle in laboratory animals. What is more important is that Lataste saw that it was a cyclic behaviour. He showed afterwards with Morau in 1895 that the behaviour cycle was correlated with an ovarian cycle, and they showed that there was a cycle in the epithelium of the vagina. All that was shown between 1890 and 1900, and I think it would be fair to recall to people that it was not Stockard and Papanicolaou in 1917 who were the first to discover the cycle of the vaginal epithelium, but that this was discovered years ago. The same with sexual behaviour All that we are doing now was done by Lataste, without hormones, of course, because he did not know what hormones were, and without experimentation, but just by observation.

We are following nearly the same methods as Lataste, just observing the individual animal It is a very important point, because in the States they are working on statistics. I have been talking about this with Richter and he agreed with me that it is much better to observe an individual animal rather than have large batches of animals and have statistics, because behaviour is an individual matter as you will see in a few minutes.

We showed first that when we give œstrogen to a female rabbit at any stage of pseudopregnancy or of pregnancy, there will be a new copulation and a new set of corpora lutea after follicle rupture.

At the start we just worked with œstradiol. We then thought that we should know which other substances are working, and we then took castrated, hysterectomised, and castrated *and* hysterectomised animals, to investigate the action of the different substances. The following technique is used : we take females which have been castrated for some weeks, and we put them together several times with males for several days. Afterwards we inject a substance and put the females back with the males, and observe the behaviour. We know exactly the number of attempts of the male to get the female, and the number of acceptances by the female. Then we have a proportion $\frac{\text{successful mating}}{\text{attempts of the male}}$ and this proportion is recorded on the ordinate of a graph, the days being recorded on the abscissa, and we then have a notation

acceptance of the male, with insemination and finally fecundation.

Thus we felt that it was worth while to study the total behaviour of the animal, and we used this point of view for a few years as a technique. The experimental investigations were performed and published with Gaston Mayer. The references are given at the end of this paper. We take the females, inject them with œstrogens in various stages of the *ovarian cycle and of pregnancy, then we put the females* together with the males, and we have follicle ruptures and other things like superfœtation and several generations of corpora lutea in the ovaries, as we like, without introducing any gonadotrophic hormones. We just put a small amount of œstrogen into the animal to raise the level, and then the female will accept. Three years ago I was fortunate enough to give here in London a paper on superfœtation obtained with this technique. Afterwards we thought that it was worth while to work out a definite technique to see how the œstrogens are working, giving the whole œstrous behaviour.

I should like to recall that at the very start of the studies on sexual physiology the first test used was the whole behaviour of the animal. I should also like to recall here the name of Lataste, who in the year 1890 knew perfectly well what we now call the sexual behaviour of an animal. I discovered *here in London in Dr. Parkes' laboratory, 15 years ago,* his book, which is absolutely invaluable, called "Principes de Zoéthique des Animaux", that is, principles of the conduct of animals. I owe many things to Dr. Parkes, and I should like to say here that one thing which I value very much is the fact that I have seen this book of Lataste. I told that to my colleagues in the States a few weeks ago, and they were surprised, I should say almost astonished, that as early as 1890 there were people who had done work on the lines that Beach and Morgan and many other people are now investigating in the States. The copy of Lataste's book which Dr. Parkes has was owned by Heape, and it is quite certain that this book inspired Heape to put forward the classification of the œstrous cycle: pre-œstrus, œstrus, post-œstrus and so on. You find all that in Lataste, without the definite terms, but all the same a very good description of all that happens

in the œstrous cycle in laboratory animals. What is more important is that Lataste saw that it was a cyclic behaviour. He showed afterwards with Morau in 1895 that the behaviour cycle was correlated with an ovarian cycle, and they showed that there was a cycle in the epithelium of the vagina. All that was shown between 1890 and 1900, and I think it would be fair to recall to people that it was not Stockard and Papanicolaou in 1917 who were the first to discover the cycle of the vaginal epithelium, but that this was discovered years ago. The same with sexual behaviour. All that we are doing now was done by Lataste, without hormones, of course, because he did not know what hormones were, and without experimentation, but just by observation.

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which is semiquantitative but which is quite useful. That is to say, we let the male have six attempts and the female can have 6 out of 6, or 5 out of 6, or 1 out of 6 acceptances, and so on. We finally have a graph which shows us very well what happens in the total behaviour of the animal.

For instance, if we take a substance like cyclœstrol, we have a curve as in Fig. 1; it is quite a general curve for one

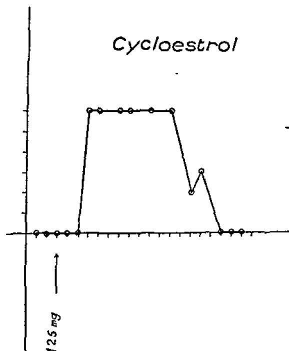


FIG. 1.
Explanation of the graphs.

On the ordinates are recorded the proportions $\frac{\text{successful matings}}{\text{attempts of the male}}$ — on the abscissa the days.

The graph gives a semiquantitative aspect of the œstrus behaviour of the female after administration of a given substance.

of the substances tried. I shall show you the curves we got, and you will see big differences in the general sexual behaviour of the female rabbit after administration of various hormones.

ll these animals are castrated and hysterectomised. In 'ig. 2 the effect of œstradiol benzoate is shown. We have nvestigated quite a number of œstrogenic substances and he results are very variable. It is very striking that when ou take a castrated female you have responses after 24 or 8 hours. You go on with the injections, but after a few days t may happen that despite the injections the female stops

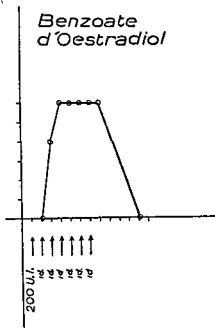


FIG. 2.

accepting the male, showing that stilbœstrol or œstradiol or any other œstrogenic substance is not able to maintain for days and days the general sexual activity of the animal. This is a very difficult problem, and we are now investigating

we often observe that once you stop the injection of progesterone, two days afterwards the female has a definite œstrous behaviour (Fig. 4). So we have a withdrawal effect of progesterone, and now have to find out what is the mechanism of this withdrawal effect. We don't know where the substance is coming from which induces some gland of the

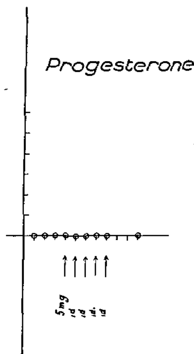


FIG 3.

animal to produce an œstrogen which, circulating in the organism, will eventually determine the sexual behaviour.

Now I come to testosterone. I should say that testosterone is a most effective substance and we now use this substance in all our experiments in this series to determine sexual behaviour (Fig. 5). We have dropped the classical œstrogenic substances and we now always use testosterone propionate or acetate, it does not matter which. The best way of doing it is to insert a pellet of testosterone under the skin

of the ear of the rabbit. It is a very convenient way of doing it because by trans-illumination we can see what happens to the inserted pellet. Here is a very typical case (Fig. 6) of a female which got a 50 mg. pellet inserted in the ear, and displayed sexual behaviour for 40 days and took the male 6 times every day. I think that it is quite a good record,

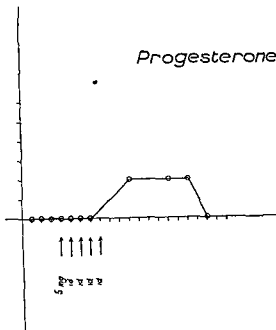


FIG. 4.

and there are quite a few of them. What is queer in this case is the fact that these females have at the same time a male behaviour. When we put such females together with females, they display quite a typical male behaviour, but this male behaviour is not quite so effective, not so typical as the female behaviour. I think this is one of the most spectacular cases. So if you want a constant, complete œstrus in females put a crystal of testosterone or of any œstrogen in the ear, and you can put this female every day with the male.

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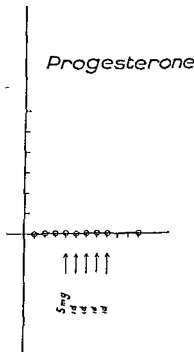


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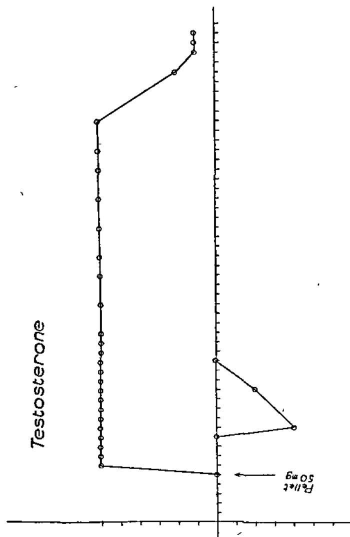


FIG. 6.

We have done these experiments with intact animals which have ovaries, and then comes a very extraordinary state, in which you find generations and generations of corpora lutea ın the ovaries with very queer responses of the endometrium. Anyhow, you see that all these substances are giving complete female behaviour.

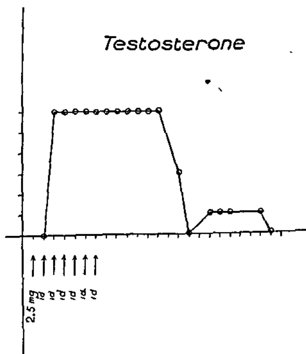


FIG. 5.

Of course, we tried to see ıf just the dilatation of the vascular system would not work equally well. We have given yohimbine to female rabbits for days and days, and the substance gave a splendid vasodılatation of the genital organs but we haven't seen any sexual behaviour (Fig. 7). So I think we can definitely drop this old question about whether sexual behaviour is just a question of vasodilatation of the sexual organs.

on very small females, say 600–700 g., of Continental strains, and can induce quite typical female behaviour.

If the ovaries are immature the female takes the male 3 or 4 times a day and there is never any follicle rupture. You have to wait until the animal is at least 1,200 or 1,400 g., to have a follicle rupture.

Often when we are in a hurry to do an experiment, and we want to put females in œstrus, especially in winter time when the animals are not in œstrus in our country, we just adopt this technique and we have very good results. As a whole I think this method is very valuable, especially for people who are working on rabbits.

I should like to add a few words on the general meaning of such statements. I should be very careful about the following point: May these results be extended to all species, and especially to man? We read everywhere that internal glands are the glands of destiny and things like that, and that gonads are the glands of sexuality. I think it is dangerous to write such things. Maybe it is all right to put that in a book if you want to sell it, but it is dangerous from the methodological point of view. It is very dangerous to assume that a substance is an œstrogen; that would mean that if you gave that substance, you have the key to the whole œstrus. That surely is not true. We have just one factor which is working, but we do not really know which way this factor is working, because I have not said a single word about the nervous system. I think that we must assume that if we give substances such as testosterone to a female, this substance is used in some way by the organism, and especially the nervous system, to induce a typical female behaviour, provided a male is present. As soon as we put this same

environment.

And there is another very difficult question. Maybe the whole soma of the animal makes a special utilization of the substance you give. I think there is no species which shows

That was the state a few months ago of our research. You see, as a whole we had to come back to the general study of sexual behaviour. The only point I showed to-day was the œstrus behaviour, but there is also a behaviour which is as interesting, or possibly more so, that is the maternal behaviour. I will not speak of this to-day, it would take up too much time. At the present moment we are doing no work on the

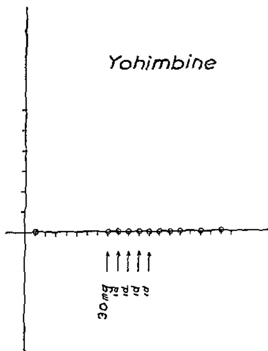


FIG. 7.

male behaviour, which is much more difficult to study than the female behaviour in the rabbit. We have with this method a technique to produce at any moment we like sexual behaviour in the female, and if it is an intact animal we get ruptured follicles and the development of corpura lutea.

I should like to add a few words concerning prepuberal or impuberal females. *We have done the same experiments*

on very small females, say 600–700 g., of Continental strains, and can induce quite typical female behaviour.

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And there is another very difficult question. Maybe the whole soma of the animal makes a special utilization of the substance you give. I think there is no species which shows

that as well as the human. I have had occasion to observe human castrates in Strasbourg. Unfortunately, there are a few the Germans had left. You probably know that under the Nazi regime there was a terrible law against sexual offences *which left the poor people charged with this offence* two solutions, either to go to the concentration camp or to be castrated. We have a few in Strasbourg who were castrated. We see them from time to time and we have applied therapeutics, giving them male hormones in various forms, either solutions or pellets, and I was not surprised to see that if you give male hormones to a male, the results may be variable from one individual to another concerning the sexual behaviour. This has been known for several years, but now we have quite typical cases of that. So I think we *must assume that even in the same sex, disregarding the* nervous system and disregarding the environment, the whole organism is using those substances in quite a definite way, either female or male.

All these questions are very difficult and it would take too much time to discuss them here.

I would like to say that if you are able to induce a complete sexual behaviour in a rabbit with a definite substance, (and there are quite a number of substances giving this behaviour and not one substance), it would be very childish to assume *that you have the substance producing* genital behaviour, sexual behaviour, œstrus behaviour, or the determination of sexual life in general.

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DISCUSSION

FORBES: Has Professor Klein any explanation for the progesterone effect?

KLEIN: Yes, there must be production of œstrogen somewhere.

ovaries are active once more. It is very queer to see a female, from whom you have taken away a complete pregnant uterus, showing first a typical maternal behaviour, plucking the hairs and sitting down on the nest and waiting for parturition that will never come, and a few hours afterwards quite a typical œstrus behaviour; you can put this female back with a male. In this case it is clear that it is the ovary which is responsible, because you have a maturation and bursting of follicles. In the castrate animal it is much more difficult to explain, but I think it is the adrenal which gives the response once you stop the progesterone. There must be somewhere a release of œstrogens, otherwise we cannot understand why a castrate female has œstrous behaviour when you stop either progesterone or pregnenolone.

FORBES: Why were the animals hysterectomised?

KLEIN: Usually these animals are the remainders of old experiments on hysterectomy. There is no difference between the animals which were only ovariectomised and the ovariectomised and hysterectomised animals.

I should like to add that these experiments call for individual observation of the animals. As Lataste did, you have to have a log book. I have had rabbits which I have been observing for years and years. There were some females which never took the male, never, for 3 years. Then I opened them and found very small ovaries, and we took the ovaries out. Then we put testosterone into these females, and got complete sexual behaviour.

BISHOP: The use of androgens to improve defective female human libido has been quite commonly practised for a good many years, but I have always understood that the rationale of that use was that it caused vasodilatation of the vulva and engorgement of the clitoris, and that it was purely a mechanical thing. However, I think what [illegible]

[illegible]

behaviour, generally attributed to the higher centres of innervation, has not been seen in your rabbits.

KLEIN: What you have said indicates that there are enormous differences between species. The rabbit is a very favourable species because you only have the provoked rupture of the follicle and the general pathway is fairly well known. In the rabbit the phenomenon is not as complicated as in other species, but is much more complicated than in the rat. So you have all gradations between spontaneous rupture, with a relatively simple procedure, and the action of the hypothalamus. I think that the action of the hypothalamus is very variable from one species to another, and the higher we go in the animal level the more complicated is the phenomenon.

FOLLEY: In cattle the question how far mere acceptance of the male is a part of the pattern of mating behaviour is rather complicated. Take sperm collection at an artificial insemination centre, for instance. You would think that you would need an œstrous female for the bull to mount, but, provided a cow will stand quietly in stocks, the bull will mount at any time during the œstrus cycle and the cow will allow that. So that I imagine that in the case of cattle, mere acceptance of the male would not be an absolute criterion of complete sexual behaviour.

PARKES: Do you use the word "accept" when you have to tie the cow up and put her into stocks?

FOLLEY: I think you will find it is justified. You get a characteristic sort of behaviour pattern which is shown by quiet cows which are kept as "teasers", which sometimes stand even without stocks.

PARKES: Yes, there are cows which stand about in a disinterested fashion, no matter what happens.

FOLLEY: Yes, but many another cow would probably not allow the bull to mount her, whereas one of these will although she is not in œstrus.

PARKES: What size squad of bucks did you have to attend to that rabbit implanted with testosterone?

KLEIN: Four or five bucks.

PARKES: Do you have to rotate your bucks?

KLEIN: Yes.

PARKES: So then it is an experiment on an individual female, but not on an individual male?

KLEIN: Yes.

PARKES: So that will make a difference when the response is half way?

KLEIN: Yes. But even in our ordinary experiments when we are quite sure we shall have a good insemination, we put the female with at least 2 or 3 males.

OVERBEEK: Does superovulation also occur in man?

KLEIN: That is a very difficult question. If you want a scientific discussion of the question, go back to the book by Grosser, who was [illegible] "d "Eihautbildung, [illegible] you will find the [illegible] ovulation, and of [illegible] ight be more able

PARKES: What you referred to was superfœtation, wasn't it?

KLEIN: Yes.

PARKES: You really have superfœtation?

KLEIN: Well, to put it more modestly, we have superimplantation. The two pregnancies go on together for 4–5 days, then the first one dies and the second one goes on We have had numerous cases, always with the same result.

PARKES: What is the difference in the age of the pregnancies?

KLEIN: Nine days.

PARKES: You haven't got them implanted in the second pregnancy?

KLEIN: The second pregnancy is perfectly implanted, and the first dies about four or five days after the implantation of the second, that is to say, on about the 15th day.

PARKES: Is the first pregnancy in both horns?

KLEIN: No, in one, because we have washed out one horn to prepare it for the second pregnancy

BISHOP: How long is the interval between the two ruptures of the follicles? Can it be right up to the later stages of pregnancy? Or is it only in early pregnancy?

[illegible] half of pregnancy, but [illegible]

BISHOP: But if it could occur only early in pregnancy, it would be extremely difficult to detect in human beings.

KLEIN: Dr. Hediger, who is a Director of the Zoological Gardens in Basle, has shown that in the wild hare superfœtation occurs quite naturally at any stage of pregnancy, even at the end This has been well known for 100 years by huntsmen and nobody believed them, but now it is quite definite The two pregnancies are not usually both living. There are just two sizes of fœtuses, and generally one pregnancy is dead.

OVERBEEK: Have you any idea why this first pregnancy is so rapidly terminated?

[illegible] generation of corpora [illegible] re is a rupture of the [illegible] you could probably [illegible] are trying pregneni- [illegible]

MACAULAY: Is the slowing down of the first generation of corpora lutea interpreted by you as a competition for circulating prolactin? If you gave prolactin would it help this retardation?

KLEIN: No, we haven't tried it yet

BISHOP: That might do it, mightn't it?

KLEIN: Yes.

KLEIN: What you have said indicates that there are enormous differences between species. The rabbit is a very favourable species because you only have the provoked rupture of the follicle and the general pathway is fairly well known. In the rabbit the phenomenon is not as complicated as in other species, but is much more complicated than in the rat. So you have all gradations between spontaneous rupture, with a relatively simple procedure, and the action of the hypothalamus. I think that the action of the hypothalamus is very variable from one species to another, and the higher we go in the animal level the more complicated is the phenomenon.

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observed in response to treatment, and the milk yield of approximately half the number of animals treated was below the level considered to be economic [i.e. 15 lbs. (6½ kg.).] This fact, together with certain side effects of the œstrogen therapy, such as persistent œstrus, precluded the wide-spread application of this technique in agricultural practice. Certain observations however, of considerable physiological interest were noted. In many cases the onset of lactation was very rapid, indicating that mammary development and milk secretion were proceeding simultaneously—physiological processes at one time considered mutually exclusive. The dosage of œstrogen and the duration of the treatment also appeared to be of major importance. Implants left *in situ* for too long, produced a fall in yield which, if not averted by removal of the tablets, soon resulted in a complete cessation of secretion. In successful cases, however, when the implants were removed at the peak of lactation, the milk yield generally showed a further subsequent rise followed by a slow decline similar to that occurring in normal lactation. The physiological significance of these observations has been fully discussed by Folley and Malpress (1948) in their "double threshold" theory of œstrogen activity in lactation.

To investigate the causes of these erratic and unpredictable responses to œstrogen therapy, various experiments on goats are in progress in Shinfield. Since it seemed likely that progesterone might well be an important factor in udder development, one series of goats were implanted with hexœstrol and a second with hexœstrol+progesterone, the total weight of the implants per animal being approximately 500 mg. of hexœstrol and 736–2,755 mg. of progesterone. The results are set out in Tables I and II. It will be seen that there is no correlation between the quantity of œstrogen absorbed and the milk yield nor does progesterone in any way improve the milk yields. The mean daily absorption of progesterone ranged from 1.7–5.3 mg., and it seemed probable that these quantities might be too small to show any physiological effect. Further experiments were therefore planned in which the œstrogen and progesterone were to be injected daily to ensure a stricter control of the dosage and to allow a high dose-level of progesterone to be employed.

ARTIFICIAL INDUCTION OF LACTATION IN GOATS BY STEROID HORMONES AND SYNTHETIC ŒSTROGENS

A. T. COWIE

UNTIL some ten years ago it was generally considered that lactation could be induced experimentally only by the injection of anterior-pituitary extracts into animals whose mammary glands had been previously developed by suitable treatments. In 1940 and 1941, however, Folley, Scott Watson and Bottomley showed that copious lactation could be induced in virgin goats by œstrogen therapy alone. When the udder region was inuncted daily with an ointment containing diethystilbœstrol, there ensued a rapid growth of the glandular tissue followed by milk secretion. Similar observations were made by Lewis and Turner (1940, 1941), who used œstrogen injection and implantation techniques.

This discovery of the lactogenic effect of the œstrogens, in addition to calling in question the then current view that œstrogens had only an inhibitory action on lactation, made available a possible hormonal method of inducing lactation in cows and heifers. Under the ægis of the Agricultural Research Council extensive field experiments were undertaken by Folley and Malpress (1944), Folley, Stewart and Young (1944), Hammond (Jr.) and Day (1944) and Parkes and Glover (1944), to ascertain whether œstrogen therapy could be employed for this purpose in sterile cows and heifers so that such animals might be maintained economically on the farm while further efforts were made to overcome their sterility. Diethylstilbœstrol and hexœstrol were used in these trials and administered either by the tablet-implantation technique, orally, or in the form of esters given at a single injection.

I shall not review these experiments in detail but only recall a few of the salient points which gave rise to the experiments about to be described. Great individual differences were

Table II

MILK YIELDS OF VIRGIN GOATS ARTIFICIALLY BROUGHT INTO LACTATION BY SUBCUTANEOUS IMPLANTATION OF COMPRESSED TABLETS OF HEXŒSTROL AND CAST CYLINDERS OF PROGESTERONE

Goat No	Age (mths)	Body weight (lb)	Weight of tablets implanted (Hx = hexœstrol Pr = *progesterone*) (mg)	Duration of implant (days)	Total hexœstrol absorbed (mg)	Total progesterone absorbed (mg)	Total milk yield over 20 weeks from *implantation* (*litres*)
117	24	74	Hx. 220, 218 Pr. 807, 795, 673, 480	107	67	562	14.1
110	18	71	Hx. 271, 271 Pr. 998	80	94	164	34.9
113	20	74	Hx. 276, 270 Pr 730	80	101	135	38.3
116	23	75	Hx. 269, 266 Pr. 1,000, 833	83	110	364	41.9
119	24	60	Hx 270, 267 Pr. 990, 601	83	130	309	13.7

Further, to rule out as far as possible genetic variations in the experimental animals, pure-bred British Saanen goatlings were used. These were ovariectomized during infancy to eliminate endogenous ovarian hormones. In this connection, however, one must remember that œstrogens and progesterone may arise from the adrenal cortex. To simulate conditions of normal mammary development it was decided that the

Table I

Milk Yields of Virgin Goats artificially brought into Lactation by Subcutaneous Implantation of Compressed Tablets of Hexœstrol

Goat No	Age (mths)	Body weight (lb.)	Weight of hexœstrol tablets implanted (mg.)	Duration of implant (days)	Total hexœstrol absorbed (mg.)	Total milk yield over 20 weeks from implantation (litres)
118	24	80	223, 204	107	66	0.6
84	11	72	477	59	74	180.8
86	11	72	493	59	89	90 8
115	23	78	269, 266	70	90	2.1
114	20	63	273, 255	80	108	36.9
120	24	74	270, 269	83	124	18.1
109	18	87	275, 275	80	141	77.4

treatments be continued over a period of time equivalent to the gestation period (i.e. 20 weeks). Since the greater part of the true mammary development occurs during the first half of pregnancy, some animals were treated for periods of 10 weeks.

The daily dose of progesterone to be administered presented a problem. No information existed on the progesterone production of the goat during pregnancy. On the basis of certain calculations by Corner (1937) on the progesterone production of the corpus luteum of the rabbit it was calculated that on a weight for weight basis the corpus luteum of the goat might secrete about 20 mg. progesterone per day. Since goats usually have 2 corpora lutea during pregnancy it was agreed that a dose of 40 mg. progesterone per day be given. I need not stress the highly speculative nature of this figure, and in view of the observations made by Dr. Forbes yesterday, this dose may well be an underestimation. The dose of

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not differ from those receiving the high dose of œstrogen (see Table IV).

The possibility that the removal of one half of the udder might lead to a compensatory hypertrophy of the remaining half was investigated in 3 goats which were in normal lactation

Table III

Milk Yields of Spayed Virgin British Saanen Goats artificially brought into Lactation by Daily Subcutaneous Injections of Hexœstrol

Goat No	Age at ovariectomy (weeks)	Age at start of injections (months)	Body weight at start of injections (lb.)	Daily dose of hexœstrol (mg.)	Injection period (days)	Total milk yield over 30 weeks from start of milking (half udder) (litres)
258	13	9½	100	1.0	70	143.1
273	8½	10	96	1.0	70	81.6
264	8	11½	94	1.0	65	61.6
323	13½	10¼	98	1 0	140	49.8
271	8½	9	100	1.0	140	36.5
283	5½	9½	94	1.0	140	17.5

and in which the milk yields of the two halves of the udder had been recorded separately from parturition. In no case did the surgical removal of one half of the udder give rise to significant alteration in the yield for the remaining half. It is therefore unlikely that this was a complicating factor in these experiments. There did occur in some cases, however, a transient fall in lactation in the few days following the operation (see Figure 1).

Brief reference may now be made to the histological findings in the udders of these goats. A careful and detailed study by Mr. Richardson, including measurements of the mean alveolar size, revealed no significant differences in structure between the various experimental groups. These

the best mammary development in the rabbit. A dose of

a

animals were paired, one receiving œstrogen alone, the other œstrogen and progesterone. At the end of the injection period milking was begun, the yield from each half of the udder being recorded separately. After the lactation curve had reached its apparent maximum, one half of the udder was removed surgically under oxygen-cyclopropane anæsthesia and immediately fixed by intravascular perfusion with Zenker's fluid. When the milk yield of the remaining half showed signs of rapid decline, usually after 30-40 weeks of lactation, it too was removed for histological examination. Typical lactation curves for a pair of goats are shown in Figure 1. I shall refer later to the histological findings but I may say here that we are greatly indebted to Mr. K. C. Richardson, who not only carried out all the histological studies but developed highly specialised techniques for preparing serial sections (100 μ. in thickness) of the whole half udder.

The milk yields obtained from this series of goats are set out in Tables III and IV. There is no significant difference between the yields of those receiving œstrogen and those œstrogen+progesterone. In the former group, the highest yields were obtained from those animals treated for a 10-week

fore be drawn from these results although they suggested that the dose of œstrogen used might be too high and this view was supported by the fact that certain toxic symptoms, such as loss of appetite, occurred in a few animals during œstrogen therapy. We have not yet had the opportunity of studying the effects of using a lower dose of œstrogen apart from two goatlings which were given 0.25 mg. hexœstrol per day in combination 40 mg. progesterone. Their milk yields did

not differ from those receiving the high dose of œstrogen (see Table IV).

The possibility that the removal of one half of the udder might lead to a compensatory hypertrophy of the remaining half was investigated in 8 goats which were in normal lactation

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Table IV

MILK YIELDS OF SPAYED VIRGIN BRITISH SAANEN GOATS ARTIFICIALLY BROUGHT INTO LACTATION BY DAILY SUBCUTANEOUS INJECTIONS OF HEXŒSTROL AND PROGESTERONE

Goat No.	Age at ovariectomy (weeks)	Age at start of injections (months)	Body weight at start of injections (lb.)	Daily dose of hexœstrol (mg.)	Daily dose of progesterone (mg.)	Injection period (days)	Total milk yield over 30 weeks from start of milking (half udder) (litres)
320	14½	10¼	92	1.0	40	140	174.1
257	8½	9¼	103	1.0	40	140	165.6
289	4½	9½	102	1.0	40	70	123.5
259	8	9¼	97	1.0	40	70	81.9
270	7½	11¼	91	1.0	40	65	76.3
287	5	9½	94	1.0	40	140	15.8
316	13	11½	88	0.25	40	70	140.7
338	13	9¾	79	0.25	40	105	84.4

results, while disappointing, are in conformity with the functional responses.

Our experiments to date have thus given inconclusive results and the role of progesterone in the growth of the udder in the goat remains a problem still to be elucidated.

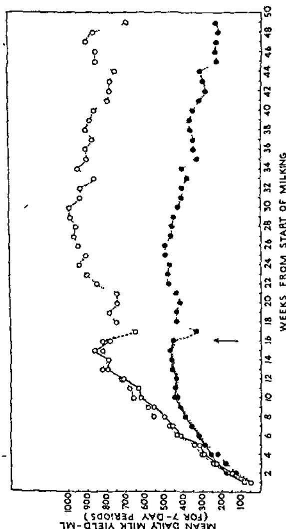


FIG 1. Lactation curves for two goats.
○ Goat 258, treated with 1 mg. hexœstrol daily for 10 weeks
● Goat 259, treated with 1 *mg hexœstrol*+40 mg. progesterone daily for 10 weeks.
Yields from right mammary glands shown in solid lines.
Yields from left mammary glands shown in dotted lines.
↑ One half of udder removed.

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DR. MALPRESS

I [illegible] I may [illegible]

upon the secretion of lactogenic hormones from the pituitary gland (Folley and Malpress, 1948). This theory was put forward *in an attempt to explain the quite extraordinary* variation in the individual milking responses of cattle receiving similar doses of œstrogen, in a way that would not conflict [illegible]

as well as inhibit lactation and it seemed that this duality of action *might best be explained by postulating a change in* the response of the pituitary to œstrogens attendant upon changes in the systemic concentrations of this hormone. In its particular application to the secretion of lactogenic hormones the theory suggests that we may distinguish three ranges of œstrogen concentration, which for convenience we may term low, middle and high, which respectively cause the pituitary to show no response, *a stimulated secretion of* the lactogenic hormones and a suppression of these hormones.

In this way there would be two "threshold" values for systemic œstrogen concentration in its relation to lactogenic activity: (i) the point at which secretion of the hormone is first evoked and (ii) the point at which the stimulation of secretion passes to an inhibitory effect.

By making the further assumption that the absolute and relative positions of these thresholds on the systemic œstrogen scale varied from animal to animal, the "double threshold" theory was able to offer a suitable working hypothesis which could explain the variations in response which were encountered in actual lactation experiments; it must be remembered however that it was, and is, a working hypothesis only and that the essential critical experiments relating blood œstrogen values, levels of pituitary secretion of lactogenic hormones and lactation responses for individual animals have never yet been carried out

A necessary extension of the "double threshold" theory has always been that a good or bad milk yield following œstrogen treatment might merely reflect a difference in the amount of mammary growth produced by the hormone—in other words, that the discrepancies are, in some cases, morphological and not functional. If this were so it might be expected that the additional provision of progesterone would complete the growth stimulus required, and that under the conditions of Dr. Cowie's experiments greatly increased and more uniform mammary growth, and lactational responses, would be given; but, surprisingly, as we have heard, this has not been the case. Dr Cowie's results would seem to place the goat, and probably we may include the cow as well, in the same category as the guinea-pig insofar as mammary growth is concerned: that is, they are species for which œstrogen alone can provide a full mammary stimulus if the dosage and duration of treatment are ideally adjusted.

The fact remains however that in experiments on the artificial induction of lactation by œstrogens there is, demonstrably, always a variation in the degree of mammary growth produced in different animals. The question of pituitary involvement in the mammary growth response is controversial; but assuming, as seems likely, that it does have some sensitizing action at least on the mammary gland

(see Reece and Leonard, 1941), one might easily find justification in Dr. Cowie's results for an extension of the "double threshold" theory, to embrace œstrogen—pituitary relationships in the control of mammary growth. It may be, in other words, that since progesterone has been shown in these experiments to have little effect on growth or milk-yield, we must now consider whether those pituitary secretions predisposing to mammary growth, as well as those responsible for lactation, cannot also be suppressed or evoked by œstrogens, depending on the systemic level of this hormone.

This wider application of the "double threshold" theory to the relation of œstrogen to hormones of the pituitary, other than the lactogenic hormones, has already been suggested elsewhere (Folley and Malpress, 1947).

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DISCUSSION

Forbes It seems to me that this stimulation and inhibition by œstrogen ties in with what I understand is the clinical procedure of administering stilbœstrol to stimulate or to check human lactation. The idea of the total œstrogen present being the important thing is a very nice concept

Brownlee. The slow and gradual preparation of the mammary gland is perhaps the most important factor. This idea gets a lot of support from what I think is generally found in the bovines. You get better results with aborted heifers than with animals which do not breed. Now the danger of drawing conclusions from the second sort is that in these infertile or sterile heifers you already have presumably an abnormal mechanism. Many of our experiments and many of some other people's experiments, but not, I think, Dr. Cowie's, were really on abnormal material. I cannot think, from Cowie's sections, that he ever got to an inhibitory level of œstrogen. Certainly in the [illegible] mammary gland before you [illegible]

with œstrogen the alveoli tended to be cystic, and when [illegible]

[illegible]

those treated with œstrogen and progesterone.

THE DIFFICULTY OF EVALUATING THE POTENCY OF STEROID HORMONES BY DIFFERENT ROUTES OF ADMINISTRATION IN HUMANS

P. M. F. BISHOP

THE literature on the clinical assessment of the potency of sex hormones and of D.C.A. by different methods of administration in humans is not very edifying, and I think the reason chiefly is that the human being is really a most unsatisfactory test animal from this point of view. Yesterday I emphasized the sort of trouble you could get into by trying to predict the daily dose from an implant because of the considerable scatter that we found with our absorption rates in most of the implants other than fused testosterone. Indeed I have not been able to discover in the literature any convincing attempt to compare the potency of these steroid hormones implants with other methods of administration. The chief difficulty, of course, is to find an accurate and not too tedious human end-point.

Now one might think straightaway that by far the most satisfactory method would be to use the urinary recovery of steroids after administration. I think Dr. Hamburger's extremely interesting and very careful studies which he told us about earlier have shown the difficulties of that. You will all appreciate very well how extremely laborious, to begin with, those investigations were. It is, I think, of very great significance that apart from a few cases of carcinoma of the cervix uteri and carcinoma of the breast where he was fortunate to have the women, as it were, [illegible] enough period of time, her on himself or else tory. He has told me he can really trust only himself to give the injection and to be quite certain that he

has got a 24-hour yield of urine. It may surprise some of you to realise that it is quite impossible in an ordinary teaching hospital which has not got a metabolic ward to get a 24-hour specimen of urine. It may seem an exaggeration, but I can assure you that it isn't so, and that any clinician who is attempting to do metabolic work of any kind without a metabolic ward would support that contention. Even so, Dr. Hamburger's results, though they were extremely interesting, I think made it quite clear that even that is not a method of comparing the relative potency of different forms of androgen, and of androgens given in different ways. One can get a vague idea of how potent one particular androgen may be, or how long the effect of another androgen may last, but one certainly cannot get a really accurate comparison of the potency of different androgens or different methods of administration. Nevertheless, there is in the literature a good deal of so-called information about this subject. It has been summarized in a very interesting and good review by Carter, Cohen and Shorr in *Vitamins and Hormones*. They have reviewed the reports up to 1947, and those reports came from nine different groups of workers. They are based on about a hundred cases only, and in the largest series, 46 cases recorded by Salmon, in which the menstrual and vaginal cycle were suppressed, there were no comparative studies on the same patient in any of the cases. The conclusions reached suggest that testosterone propionate by injection is about three times as potent as methyl testosterone by mouth, but the evidence is so poor that I don't think that that statement means anything at all.

Personally I don't think we have any idea at all of the relative potency of methyl testosterone by mouth compared with testosterone propionate by injection.

Now, taking D.C.A. From the point of view of urinary metabolic studies, you all know how difficult that is. It is probable that there is a slight rise in the pregnanediol output in the urine after the administration of D.C.A., but there is no very good evidence of anything like a deoxycortone coming out in the urine itself.

With regard to progesterone, we are now fairly certain of two things. One is that in order to get really accurate

estimations of pregnanediol you are committed to an extremely laborious method. I think that has been thoroughly emphasized recently by Marrian. Furthermore, I think it has also been shown fairly conclusively that the amount of exogenous, administered progesterone which is excreted as pregnanediol is variable, and therefore the pregnanediol levels are not an accurate indication of progesterone activities. Thus, it seems quite impossible to compare the relative potency of progesterone by injection and of ethisterone by mouth. That, however, has not prevented people from making statements about it. I shall give you the evidence, such as it is, which exists at the present moment. Inhoffen and Hohlweg (1938) when they first introduced ethisterone found that roughly four times as much oral ethisterone was required to induce secretory changes in the uterine mucosa of the rabbit as parenteral progesterone.

Buxton and Engel (1949) state that ethisterone by mouth has about one-sixth to one-tenth the potency of progesterone by injection. Wiesbader (1941) puts the ratio at 1 : 6 or 1 : 7. Joel (1942), using the production of secretory endometrium in menopausal women, previously primed with œstrogen, as a criterion, gave a ration of 1 : 5–8, if the ethisterone were [illegible]

ratio at 1 : 6 to 8. Selye (1947) states that the ratio is 1 to 5, and the Council of Pharmacy and Chemistry of the American Medical Association doubted whether clinical studies had convincingly proved that oral ethisterone will accomplish all the effects obtainable by progesterone. So that's the position as far as the progestogens are concerned in the human being.

What other possible methods might there be of assessing in the human being the relative potency of these different steroids administered by different methods? I was very interested in Dr. Overbeek's remarks yesterday about D.C.A. I asked him afterwards what particular clinical criterion had been used to demonstrate the efficacy, or cessation of efficacy after D.C.A. administration, and he told me that

there was nothing very accurate about it—it was merely that some patients with Addison's disease were definitely able to say whether they were better or whether they were worse. Although I would agree with him from my clinical experience that that is the case, it is hardly good enough even for a clinician to pin any faith on relative potencies from something quite so subjective as that.

I am coming to the conclusion that it is very hard to demonstrate the effect of progesterone in the human being at all. One can produce a progesterone withdrawal bleeding in women who have been primed with a constant dose of œstrogen, but that progesterone withdrawal bleeding is so variable and depends on so many various factors, such for instance as the degree of œstrogen priming, that I think it may prove difficult to use this as an accurate method of assessment of relative potency of, say, progesterone by injection and ethisterone by mouth in the human being. And indeed, I am coming very much to the conclusion that we really have not the remotest idea what is the therapeutic dose of progesterone. Not many years ago we used to talk of one rabbit unit, that is, 1 mg., as being the therapeutic dose. Now we believe it is rather more than that. Some people say 5 mg., some people say 10 mg., some people are giving 25 mg., and people are even beginning to use 60, 100 and we, for experimental purposes, have used 300 milligrams of progesterone a day. The interesting thing about the use of the 300 mg. progesterone a day, in our experience, is that it does [illegible] e the [illegible] mild anæsthetic effect, such as one sees when one gives progesterone intraperitoneally in the rat. However, it does not seem to upset the menstrual cycle in women given 300 mg. progesterone a day.

With regard to androgens, what other possible methods of assessment might there be in human beings? It is perfectly true that adult castrates, men who have been castrated in adult life, either as a result of tuberculous epididymitis or war wounds, do usually develop hot flushes. These hot flushes may be very frequent indeed, and it is just conceivably possible that one might use the hot flush count in adult

castrate men as the end-point for androgen evaluation. But fortunately we haven't got sufficient adult castrate men to make it possible to do that on the sort of scale that would be necessary to satisfy a statistician with regard to our results.

The [illegible]
in th [illegible]
defici [illegible]
is only one group of workers who have claimed that they have been able to follow changes in the urethral smear of the male, and other groups of workers have tried to repeat this work and have been unable to confirm it. If it were possible, then, I suppose the urethral smear might be used as a reasonable end-point. It is a method I personally have not gone into at all. I am not certain how possible it would be to gain the co-operation of enough men to do urethral smears on them or to get them to do urethral smears on themselves. Therefore, it does seem to me that as far as androgens are concerned there is a considerable amount of inherent difficulty in trying to assess these compounds in the human being at all.

When we come to œstrogens we find that the literature is equally confusing. I would like to give one example of that, and that is the statements that have been made with regard to the administration of stilbœstrol intramuscularly and orally. This is very much on the same scientific level as that concerning the progesterone. Thus, Salmon, Geist and Walter (1940), using 45 menopausal women, employed doses which were in the ratio of 1 intramuscular to 2 or 3 oral. Huberman and Colmer (1940), using 77 menopausal women, employed doses in the ration of 1 to 6. McBryde, Freedman, Lœffel and Castrodale (1940), using 73 menopausal women, employed doses in the ration of 1 to 2. Mazer, Israel and Ravetz (1941), from their experience with 189 cases, found that the ration was 1 to 5 (this was based on the production of œstrogen withdrawal bleeding in 19 out of that 189 cases). Felger and Gendel (1940), in 20 menopausal women, comparing oral stilbœstrol against parenteral œstrone in 16 cases, and parenteral stilbœstrol against parenteral œstrone in 4 cases, obtained comparisons from which the parenteral-oral stilbœstrol ratio can be calculated as 1 to 2. Hoffmann

(loc. cit.) states that stilbœstrol is estimated to be one-half to one-sixth as effective by mouth as by injection in the human.

With œstrogens I think there is the possibility of devising human end-points rather more easily than there is with the other steroid hormones. For instance, one could quite easily imagine that one might assess it on vaginal smears in menopausal women, but in practice the vaginal smear technique in the human has not proved to be very satisfactory for such assessments. Many menopausal women show perfectly *normal œstrous smears, and, even if they do show an atrophic* smear, it is sometimes not sufficiently labile. That is to say, it is not possible to convert the atrophic smear into an œstrous smear in a short enough period of time, and then let the woman come down to a base line with an atrophic smear, and then use another dose, or another compound and bring her up to an œstrous smear, and go on doing that until one compares two œstrogens in the same woman in a short enough period of time.

As for the inhibition of lactation, which has been reported on in a good many of the clinical papers, and from which deductions have been made with regard to the relative potency of different œstrogens, it is something that you can only do once. Either you do, or you do not inhibit the lactation in any one individual woman, and you cannot compare two œstrogens on the same person.

We have found, however, that by using amenorrhœic women and producing œstrogen withdrawal bleedings, we are able to obtain what we call a therapeutic unit, that is to say, the mean between two close doses, one of which does produce [illegible] which does not. Thus, [illegible] ne œstrogen on the same [illegible] seem to stand up to statistical analysis. In that way we have been able to compare the relative potency by mouth of stilbœstrol, dienœstrol, hexœstrol, ethinyl œstradiol, bisdehydro-doisynolic acid, and at the present moment we have embarked on a further comparison of stilbœstrol sulphate, œstrone sulphate, hexœstrol sulphate, dienœstrol sulphate, pure œstrone and equilin. Provided that nobody discovers a satisfactory cure for amenorrhœa in the meantime, we hope we may possibly have some figures within the next five or six years!

As far as I can see at the moment, that is the very unsatisfactory state of any attempts to attain a clinical assessment of these steroid hormones by administering them in human beings.

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DISCUSSION

BISHOP: What did you do? Just take a smear?

OVERBEEK: Yes.

BISHOP: It might be possible.

OVERBEEK: I have looked up my notes on the activity of D C.A. in patients, and the physician has always used the diastolic pressure as a criterion

BISHOP: That is something objective, but I would personally doubt whether changes in the diastolic pressure are sufficiently stable to pay very much attention to

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œstrogens this difference between experimental and clinical potencies is most discouraging.

We were thus faced with the problem of finding a test animal which would give the same answer as that obtained clinically. We therefore re-examined most of the tests described in the literature, but unfortunately, the results obtained were similar to those obtained by our original technique using ovariectomised rats, and none agreed with the clinical results.

Very little use, however, seemed to have been made of the observation by Parkes, Dodds and Noble in 1938 that pregnancy in the rat and rabbit could be interrupted by synthetic œstrogens, and we attempted to develop an assay method using the interruption of pregnancy as the end point.

Adult female rats were examined at intervals and those in œstrus or pro-œstrus were mated and examined for vaginal plugs. Those in which successful mating had occurred were divided into groups and dosed with graded doses of stilbœstrol and ethinyl œstradiol. They were killed after 8 days, the uteri examined for implants and the percentage of pregnancies in each group recorded.

The results obtained are shown in Table I. Very few animals were used in this experiment and consequently the results are only very approximate. The E.D.50 for ethinyl œstradiol would appear to be about 60 μ/gms. and that of stilbœstrol about 9 μ/gms.

It is clear, therefore, that ethinyl œstradiol is very much less active than stilbœstrol, possibly about one-sixth as active. As clinically it is 20 times as active as stilbœstrol, this method is clearly useless as a reliable method of assay and was not studied in more detail.

To summarise, the position briefly is this :—

The rat vaginal smear technique for the assay of œstrogenic substances will give results which are reproducible in different laboratories.

The biological results may or may not agree with the clinical results and consequently the investigation of a new œstrogen should include a properly controlled clinical test.

We can offer no explanation of the discrepancy between biological and clinical findings ; it may be due to differences

OBSERVATIONS ON THE RESULTS OF PHARMACOLOGICAL ASSAY OF SYNTHETIC ŒSTROGENS AND THEIR CLINICAL EFFECTS

W. A. BROOM

In the previous paper Dr. Bishop made the point that the human being is an unsatisfactory animal from the test point of view. On the face of it the rat appears to be a much better one because the results obtained from an assay can be statistically analysed and the limits of error obtained are usually very small. Unfortunately, these results are sometimes quite different from those obtained clinically.

For some time now we have been using the ovariectomised rat as a test animal for assessing the œstrogenic activity of both natural and synthetic œstrogens and until recently have always assumed that the results obtained would agree with the clinical findings. This view was confirmed by Dr. Bishop who found hexœstrol and dienœstrol to have on patients 25 per cent and 6 per cent the activity of stilbœstrol whilst our figures on rats were 68 per cent and 10 per cent. Although the clinical and biological figures are not identical they are at least of the same order. When, however, Dr. Bishop and I both tested some further compounds, the findings were completely different, thus we found 7-methyl-*bis*dehydrodoisynolic acid to have 5 times and ethinyl œstradiol one-fifth the activity of stilbœstrol, whilst Dr. Bishop's figures respectively were one-fifth and 22 times. In other words, we found on the rat that the doisynolic acid compound had 25 times the activity of ethinyl œstradiol, [illegible]

point of view of the research worker looking for new

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The biological results may or may not agree with the clinical results and consequently the investigation of a new œstrogen should include a properly controlled clinical test.

We can offer no explanation of the discrepancy between biological and clinical findings ; it may be due to differences

in the rate of absorption from the rat and human stomach or to different rates of inactivation in the livers of the two species. I am hoping that in bringing these results to your notice this afternoon some one may put forward some suggestions which may help to develop a method of assaying *œstrogens which will give a result which will agree with* clinical findings.

Table I

Comparison of Ethinyl Œstradiol and Stilbœstrol
Rat Pregnancy Technique

Oral Dose μ gms	Proportion of Pregnancies		
	Ethinyl Œstradiol	Stilbœstrol	No Treatment
192	0/5	—	—
144	1/5	—	—
48	2/2	—	—
36	3/3	—	—
24	3/3	0/2	—
12	2/2	0/2	—
10	—	1/3	—
9	—	4/8	—
8	—	2/3	—
7.5	—	1/2	—
6	2/2	7/8	—
3	2/2	—	—
1.5	2/2	—	—
0	—	—	14/14

It should, I think, be emphasized that this discrepancy was only brought to light because Dr. Bishop had succeeded in designing a satisfactory method of clinical assay. His assay technique was so designed that the results could be subjected to statistical analysis and the limits of error calculated. I am sure the experimentalist is deeply indebted to Dr. Bishop for devising such a reliable clinical assay technique.

DISCUSSION

[illegible] ences are inherent differences [illegible] due to different metabolism, [illegible] distribution in the body in

BROOM: I thought at one time that the discrepancy was due to different rates of absorption and or destruction, because when you compare the actual doses which produce a 50 per cent response, for 7-methyl*bis*-dehydrodoisynolic acid they are the same orally and subcutaneously; whereas for ethinyl œstradiol the oral is 125 times the subcutaneous dose. One wonders whether this is due to the poor absorption or the increased destruction of the ethinyl œstradiol

BROOM: You're thinking about vaginal work?

PARKES· I was going to ask whether Broom tested these compounds by the vaginal test, which cuts out both absorption and metabolism.

BROOM Williams has used that method and he seemed to think it wasn't much good. The results weren't very accurate

PARKES Emmens was most successful with it

GADDUM Dr Bishop should do local application to the human vagina too, if you're going to get real agreement.

BISHOP I did talk to Emmens about this at the very beginning and we found that his results didn't correspond with mine.

GADDUM· That may be the fault of the human beings in absorbing it and distributing it in a misleading sort of way It would be interesting to know whether there really is a difference in the sensitivity of the tissues themselves.

BISHOP. I ought perhaps to have emphasized that this test is only at a certain œstrogenic level, that is to say, amenorrhœic women producing œstrogen withdrawal bleeding. We may find that the

BROOM· Did the work on fowl give any indication of the ratios of the synthetic œstrogens?

*Emmens, C. W (1950) *J. Endocrinol.*, 6, 302.

PARKES: No. I was dealing only with the effect of method of administration.

that your table shows that dimethyl- and tetramethylstilbenes were more active by mouth, by whichever mechanism they were studied, than they were by parenteral injection. In our experiments the tetra- and the dimethyl stilbenes had about the same activity parenterally and if added to the diet. If given by stomach-tube they were less active, an effect attributed to the rapid stomach-emptying time of the rat.

Another interesting thing is that it appeared to be absolutely essential to establish a dose-response curve for *each* route of administration of *each* œstrogen, because the slopes vary from 14 to 36. If you did not know the precise dose-response curve and assumed a straight line, large errors would be introduced.

One other point is that the pH of the rat's stomach is considerably different from the human stomach.

FOLLEY: Miss Josephine Barnes claimed that dienœstrol was more active than stilbœstrol, given by mouth, in the human. Has there been any more work on this question?

BISHOP: I think there are only two more papers, so far as I can find, in the literature about the activity of dienœstrol in comparison with stilbœstrol.

GADDUM: Do the results vary greatly between individual women?

BISHOP: No. Emmens did the statistics on this first paper for me, and he told me that the method compared with a biological assay using about 40 to 60 animals

or

an hundred-fold range?

BISHOP: Over a two-fold to ten-fold range. Certainly not more than ten.

GADDUM: It's rather remarkable, when the absorption from the intestine probably depends on so many different factors that you've

the
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d to

*Brownlee, G., and Green, A. F. (1947). *J. Endocrinol.*, 5, 158.

CLINICAL IMPRESSIONS OF VALUES OF ŒSTROGENS AND ANDROGENS ADMINISTERED BY DIFFERENT ROUTES

G. L. FOSS

I WOULD emphasize from the start that these remarks are merely clinical impressions and, as such, they lack the scientific backing that all the preceding papers have had. Although they go back to the inception and the introduction of both œstrogens and androgens to therapy, they are limited to the experience of about three thousand cases, owing to the intervention of the war years

Before the introduction of synthetic œstrogens the field of œstrogen therapy was restricted to that of œstradiol benzoate by injection. That obviously had certain advantages, in my opinion. Firstly, the wholesale and indiscriminate use of œstrogens as occurs to-day was impossible, and was limited by the price. Looking back, it is difficult to recollect any patients who had toxic effects induced by injection of œstradiol benzoate. One was able too, in those days, to give big enough doses to have an effect on, for example a hopeless inoperable fungating carcinoma of the breast. I [illegible]
n [illegible]
fc [illegible]
1940 in this country, to the use of ethinyl œstradiol by mouth. I had used it about the same time as stilbœstrol was introduced, but in my opinion, it was extremely toxic, and I used stilbœstrol instead. At present one has a choice of œstrogens and routes of application which are suitable to cover the whole range of treatment, in addition allowing for the sensitivity of the individual patient and for the particular condition for which it is required. The question of toxicity of the œstrogens, particularly ethinyl œstradiol and stilbœstrol,

is extremely difficult to decide. In some respects it depends on the potency of the particular œstrogen, but there are certain factors which suggest that that may not be the whole story. There is some clinical evidence that the synthetic œstrogens differ in minor degrees, for example, on the type and degree of pigmentation of the nipple and the areolæ. The colour from ethinyl œstradiol is different from that of stilbœstrol.

My experience with stilbœstrol is that in the normal *therapeutic doses employed it does produce toxic effects* in about 40 per cent of cases. There are various assessments of this, and some people d[illegible] hand, if one persists with [illegible] high dose, the patient ma[illegible] some never can take any synthetic œstrogens, and in those cases one has to use œstradiol benzoate. Even ethinyl œstradiol in doses as small as 0.01 mg. has produced toxic effects in some cases. On the whole I think it is fair to say that synthetic œstrogens more often cause toxic effects in the pre-menopausal female, and that may limit adequate therapy.

However, accepting the disadvantages of synthetic œstrogens there is no doubt that both for the patient and for the doctor oral administration is a considerable advance in therapy. I think the important thing is the ease of control, but it does depend on having an intelligent and co-operative patient. Oral therapy, in small or moderate doses, is effective in the treatment of most cases of menstrual disorder, where it is necessary [illegible] The tendency now is to red[illegible] conjunction with ethisterc[illegible] one in the proliferative [illegible] he secretory phase, or both together as in the Zondek technique.

Again, if the patient can tolerate it, oral stilbœstrol is extremely useful in the treatment of menorrhagias, if given in necessary doses, rapidly raising the dose well above the threshold.

Similarly, it is the main treatment for carcinoma of the prostate. In most cases adequate control can be maintained for long periods by doses of, say, 20 mg. of stilbœstrol daily.

It is also useful in advanced cases of mammary carcinoma, particularly in the older, post-menopausal patient with the rather slow-growing scirrhus carcinoma. On the other hand, one may require very much bigger doses in these mammary carcinomata, even up to 200 mg. a day before a definite effect is achieved. Using such doses, if tolerated, I have seen pleural effusions absorbed, and secondary skin deposits and glandular metastases clear up, and in the cases on which biopsies have been done, the growth has been replaced by fibrous tissue. It is interesting to note that in these old post-menopausal cases of mammary carcinoma, as long as they continue to take the high dose, say 20 mg. a day of stilbœstrol regularly, they very rarely show withdrawal bleeding. Withdrawal bleeding when it occurs is due to the fact that they have, for some reason, reduced their dose. Another thing that one notices in these patients is that they seem to acquire some tolerance to large doses of stilbœstrol, or else some increased inactivation occurs, because excessive pigmentation of the nipple and keratinization may become less or disappear in the course of time.

In males with carcinoma of the prostate, I think the incidence of toxic reactions is very much less than in females. In general, the use of these oral œstrogens is limited by their toxic effect.

Œstrogen Injection. Sometimes a better clinical result can be obtained by giving injections of œstradiol benzoate, particularly, as Dr. Bishop mentioned, for treatment of primary ovarian agenesis, where one wishes to develop the secondary sexual characters. Most of these people are disturbed mainly by their lack of breast development. I have certainly got better results in those cases by giving combined injections of œstradiol benzoate and progesterone, in doses of 5 mg. of the one and 25 mg. of the other, than by giving quite large doses of stilbœstrol and about four to five times as much ethisterone.

[illegible] I think that this route of [illegible] Again, I think Dr. Bishop [illegible] with an intact and normal-sized uterus extremely profuse bleeding may occur, from 80 to 90 days after. In some respects that can be controlled

by rhythmic application of progesterone. I think that implantation of œstrogens is limited to cases of primary ovarian agenesis where there is a tiny uterus or, as Dr. Bishop said, to cases of menopausal women who have had a hysterectomy. Then probably a small dose of the order of 20 mg. is sufficient. In advanced breast carcinoma implantation might possibly be rational therapy, but there again one is limited by the difficulties and upset of severe bleeding when the level of œstrogen dwindles by absorption of the tablet.

In males. A series of about a dozen cases of severe acne, stimulated by a demonstration of cases by Bishop at the Royal Society of Medicine, has been treated by implantation of 200–300 mg. of either stilbœstrol or œstradiol, either by a single or repeated implantations. Subsequent follow-ups have made us feel that it is an extremely hazardous therapy. Impotence occurs in most cases, and is very prolonged. More serious, however, is the effect on the tubules; azoospermia and oligozoospermia are usually found. I must admit that so far in the follow-up I haven't found return to a normal semen picture. It is quite possible that there may be permanent damage to spermatogenesis Another controversial point is the treatment of male homosexuality. For that very reason, for patients who desire such treatment, I think the implantation of stilbœstrol or œstradiol is extremely valuable and the effect of stilbœstrol lasts from four to six months, and œstradiol a year or longer.

Implantation has been used in six cases of carcinoma of the prostate. They had repeated implantations, every four to six months up to about two years. Out of this series I have only one case alive, but during that time treatment was perfectly adequate, as assessed by their clinical findings and also by the level of the acid phosphatase of the blood.

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Turning to androgens, the greatest advance in this therapy was the discovery that methyltestosterone was active by mouth. For a large number of cases—one might say almost the majority of cases—oral methyltestosterone is, in my

experience, perfectly adequate. Weight for weight, one has to use more methyltestosterone than testosterone by implantation or testosterone propionate by injection, but the relationship between them may, however, be very much lower, certainly lower than my original assessment in 1938 of 20 : 1, methyltestosterone to testosterone propionate. In a eunuch a maintenance of 30–40 mg. daily is sufficient to insure potency with emission. I have several patients with primary testicular agenesis who, after full penile development by testosterone propionate or implantation have then been maintained for various reasons perfectly adequately on 30–40 mg. per day of methyltestosterone by mouth. They have since married and can function perfectly normally.

I think the chief value of oral methyltestosterone therapy is its simplicity. It certainly removes the burden of injections, and the dose can be increased, decreased, or withdrawn at will. One can't do that with implantation. If bigger doses are required implantation has to be repeated, and it's sometimes quite difficult or troublesome to remove tablets, if necessary. It is cheaper, weight for weight, than testosterone propionate or testosterone tablets for implantation (it's about four times cheaper than testosterone tablets for implantation) but obviously one has to use 4 or 5 times as large a dose, so it works out about equal.

Certainly I think oral therapy is adequate in the following conditions: for example in small boys with hypo-or epispadias for preliminary development of the penis to enable the plastic surgeon to have a better field for operation; stimulation of puberty; stimulation of growth; continued substitution therapy; Simmond's syndrome; certain cases of menorrhagia; and some cases of fibro-adenosis of the breast.

Another advantage of methyltestosterone therapy is that, especially with small boys above mentioned, one can start with a very small dose, and work up. To my mind, it is much more physiological to start with 5 mg. a day, or even less, and gradually build up until one gets an effect, rather than to produce a dramatic response by giving injections, as was so common in the early publications. With *implantation also, as we have heard at this meeting, one* gets a surge of absorption to start with, which may be quite

by rhythmic application of progesterone. I think that implantation of œstrogens is limited to cases of primary ovarian agenesis where there is a tiny uterus or, as Dr. Bishop said, to cases of menopausal women who have had a hysterectomy. Then probably a small dose of the order of 20 mg. is sufficient. In advanced breast carcinoma implantation might possibly be rational therapy, but there again one is limited by the difficulties and upset of severe bleeding when the level of œstrogen dwindles by absorption of the tablet.

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In my opinion other methods of œstrogen therapy, such as inunction, suppositories, vaginal capsules, etc., are probably worthless, or very rarely required. Certainly I have limited experience of them.

Turning to androgens, the greatest advance in this therapy was the discovery that methyltestosterone was active by mouth. For a large number of cases—one might say almost the majority of cases—oral methyltestosterone is, in my

source. During the war it was reported, first of all by Scarff and Smith (1942) that various workers in the factories where they were making synthetic œstrogens were developing gynecomastia and it was believed that these effects were due to inhalation. Therefore, some work was undertaken at Porton by Professor Gaddum and myself using male guinea pigs to find out whether synthetic œstrogens were more effective by mouth or by inhalation.

The method of assay was the development of the male guinea pig's nipple. The nipples of the anæsthetised animals were measured and photographed against a scale. Batches of 10–20 animals were used. One group was given 1 per cent solution of hexœstrol in arachis oil by mouth, and the second group was exposed to an atomised spray of the same solution of hexœstrol. The animals were photographed at intervals on the 6th and 14th day. Calculations were based [illegible]

nipples as well as enlargement. Allowing for the difficulties of assessment it was our opinion that hexœstrol by inhalation was 5 to 10 times more potent than hexœstrol by mouth.

The idea of resuscitating this apparently impracticable method of application of synthetic œstrogens suggested itself to deal with the difficulties of coping with the large doses used for example, in carcinoma of the prostate. There one finds with the passage of time that the patient does not react to therapy and one has to increase the dose to obtain any clinical relief and to control the enormous rise in the acid phosphatase. In my cases I have given up to 1,000 mg. stilbœstrol a day over considerable periods of time, and yet the patients have continued to go downhill. One is limited in giving these large doses orally partly by the toxic effect and partly by the difficulty of giving large numbers of tablets by mouth. If one could give this therapy by inhalation, and if in man the same relationship occurs between inhalation and oral therapy as in the guinea pig, this treatment would be worth while. It would be impossible to assess the dose accurately, but in these hopeless cases that would be of little importance. Is it possible to put up this enormous

unpleasant, and the patient may sometimes complain, especially in a young subject, of considerable priapism.

The only limiting factor with methyltestosterone is time. Where a more rapid or powerful effect is required one obviously has to use the other methods of implantation or injection.

Implantation is valuable where one cannot be sure that oral therapy will be adequately supervised. Implantation, although simple, certainly does involve the use of full surgical technique, and has to be repeated every four to six months.

The only practical method achieving a rapid and powerful androgen effect is by injection of testosterone propionate, and this is the method of choice where very large doses, such as are used for carcinoma of the breast, are required. It is too early yet to assess results in this controversial subject, and I have very limited experience with liberal doses, only 20 cases. These patients have been given 300 mg. a day of testosterone propionate for weeks or months until it is evident that it is having no clinical effect and the patient is obviously practically moribund, or until there is a definite clinical improvement. Then the dose is reduced to 200 mg. on alternate days or twice a week, or something of that order. Two patients have received 40 g. altogether, and one of them has received 60 g. Using these enormous doses, I have so far only succeeded in keeping two patients alive for fifteen and a half months. Admittedly, they were practically moribund when first seen, riddled with secondaries, and had been discarded by surgeons and radiotherapists. But it does make one wonder whether this therapy is worthwhile, owing to its enormous expense. Here I should express my indebtedness to Ciba, Organon, and B.D.H. for the supply of some of these enormous doses.

I think the application of the micro-crystal suspensions might be valuable for this purpose, but I haven't had any experience of that.

Other methods of application for androgen therapy I think are extremely limited and have not much value.

Administration of Œstrogens by Inhalation

This is an application of hormones which has, as far as I know, not been confirmed or published, apart from the one

FORBES: When you used the nasal spray did you have any cases where there was congestion of the nasal mucosa?

FOSS: I have not done any work on humans, but only experimental work on guinea pigs.

FORBES: You mentioned primary testicular agenesis. I understand that this is a rare condition.

FOSS: I think that it is relatively common with variations of the syndrome.

BISHOP: I have seen a case of Turner's syndrome in the male.

With regard to the dose of androgen given in carcinoma of the breast, as Dr. Foss said, it is extremely costly treatment, and therefore, it is very important to try and determine what is the minimal effective dose of androgens in these cases. There was a very important con- [illegible] unnecessarily high dose.

FOSS: It probably is. I was only using 300 mg. a day to see if in certain cases I could produce effects not produced by smaller doses. Of course, that is about the limit to which one can go, as it produces toxicity in about 100 per cent of cases.

dose? Based on the original calculations in our paper, and assuming, for example, the use of a 1 per cent solution of hexœstrol, patients breathing deeply at about 10 respirations per minute would inhale about 1,000 cc. at each breath, which is 10 litres per minute. One would therefore inhale about 100 mg. of solvent a minute, containing 1 mg. of œstrogen. It would obviously be impossible to put up an adequate dose of hexœstrol, and stilbœstrol is less solvent, but would it be possible to put up ethinyl œstradiol in some suitable solvent? I have no idea of its solubility or of a suitable solvent for the purpose, but I suggest that it might be done for these hopeless cases. I have brought up this subject in order to get your opinions.

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Scarff, R. W., and Smith, C. P. (1942). *Brit. J. Surg.*, 29., 393.

DISCUSSION

[illegible]

[illegible]

for the treatment of acne.

Gross: Do you use œstrogens for acne only in males?

Bishop: No, also in females.

Foss: I have not had good results from œstrogens in acne in women.

[illegible]

feeling that hens may not be the same as mammals, and that possibly the results obtained in that way may not [illegible]

was an interesting example of how one can use physiological effects to study physical facts.

Another way of studying absorption is by estimating the concentrations in the blood or in the urine. Dr. Forbes' paper was a remarkable demonstration of what can be done in that way, and I hope that, in time, we will be able to study many other hormones in the same way. By estimating the concentration in the blood you do get a direct estimate of the amount of hormone to which the organs are exposed. You do not know how much the target organs actually contain, which is the thing you would like to know most, but you are rather nearer to it than by any other method. Estimations on the urine, as has been emphasised by various people, can be very misleading. You may easily read more into them than they will really stand, but it seems to me that they are often worth making, in addition to [illegible]

want to know both things

The other way of studying absorption is by the amount left in the depot, and Dr. Forbes and Dr. Folley both told us about the results obtained by that method It can be very beautifully studied when you use an implanted tablet and take it out and measure it, but with most other forms of injection it is much more difficult to find out how much is left. People have injected bismuth and then taken X-rays to see how wide is the distribution of the drug at the site of injection, and followed the absorption by taking X-rays at different times. Others have used radioactive injections and then brought a Geiger counter near the place to see how much is still left after various times. I suppose the danger of using that method to study the absorption of steroid hormones is that the steroids might be all gone and the label may be left behind to give you records on the Geiger counter.

CHAIRMAN'S CLOSING REMARKS

J. H. GADDUM

I PROBABLY know less about hormones than anybody else in the room. What I can do is to try to remind you that the question of absorption and fate of hormones in the body is really part of a larger problem of the absorption and fate of drugs in general.

Until quite recently the only method of administering drugs was oral administration. Primitive man must have gone around eating practically everything he saw, and he discovered a number of drugs by that method, including many purgatives. Purgatives are the most plentiful things in our materia medica museums. It is very easy to observe their effects. It always seems remarkable to me that drugs can produce any appreciable effect when taken by mouth. They are first exposed to strong acid, then to strong alkali, then to a collection of powerful enzymes, and then, when they get absorbed, they have got to get past the liver, a thing that anybody can be proud of getting past! The liver can destroy most things.

Absorption by skin is a method, of course, which has been practised for a long time. People used to rub mercury into the skin, but it is an unsatisfactory method and, I imagine, except for getting local effects it won't have much permanent application.

So that the important methods really are injections and, allieb methods. The effects of drugs given by injection or by implantation can be modified by affecting their rate of absorption.

There are various methods of studying the rate of absorption. The most popular method is to study their effect in the animal, and we have heard many examples of studying the effects of steroids on the animal and comparing the different methods of administration. Dr. Parkes gave us a very beautiful example, right at the beginning, but I have a

feeling that hens may not be the same as mammals, and that possibly the results obtained in that way may not necessarily be applicable to other animals. We saw some ni[illegible] de[illegible] we[illegible] effects to study physical facts.

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and causes much of the steroid to be left behind. The same thing applies to globin insulin. That is a solution which is precipitated in the tissues; or again bismuth tartrate which is precipitated in the tissues; in that case it is more rapidly absorbed than oily bismuth mainly because it is more widely distributed at the original site of the injection, so that the area of absorption is bigger.

There are also oily solutions and we have had various examples of them. We have heard about the use of various insoluble esters of steroids, which enormously prolongs their action, but that is rather an old story, and I suppose that is why we have not had more emphasis laid on it to-day, though we have had many examples of it. We have not heard very much about the effects of the nature of the oil. I believe it is true that if you add bees' wax to oil you make it more solid and less water-soluble, and delay the absorption of substances which have been dissolved in it.

Then there are suspensions. We have heard very interesting things about the effects of crystals and a good deal of evidence that absorption does depend on the size of the crystals in the suspensions. You can, of course, also have suspensions in oil. I imagine that that does not work with steroids because, of course, they dissolve in oil. But people have used suspensions of adrenaline in oil in order to get prolonged adrenaline-action, and suspensions of histamine in oil have been experimented with in order to get a prolonged histamine-action.

And then, of course, there is implantation. I feel that most of the techniques for delaying absorption have been devised by the endocrinologists, and the pharmacologists ought to be very grateful to the people who discovered implantation. But I have not been able to think of any practical application of that outside the steroid field. It is a beautiful technique and of great general interest.

The other thing about which I want to say a little is the duration of the action of the drug after it has been absorbed. Some drugs, like phenolphthalein, for example, which get re-absorbed and excreted again, are liable to go on working for several days because they undergo this circulation in the body. It makes them rather uncertain in their action.

I was very interested in what Dr. Gross told us about the comparison of the rate of absorption of tablets in animal tissue and *in vitro*. I should like to have heard more details about that and to know whether the two methods always give comparable results. It seems to me that if that is a reliable method of plotting the rate of absorption of tablets, we will in time use no other method. Perhaps it is not always very reliable.

Consider now the factors which affect the rate of absorption. We have not heard much about the site of injection, but I think it is generally agreed that subcutaneous injections are absorbed slowly, and that intramuscular ones are absorbed more quickly. We have not heard very much about the methods by which drugs are absorbed. There is evidence that it depends on the molecular weight. I do not know whether you know anything about the experiments of Barnes and Trueta, who injected various substances, such as snake poisons, and found that substances with molecular weights over 20,000 were absorbed mainly by the lymphatics, whereas substances with molecular weight less than 20,000 were absorbed mainly by the blood vessels, and that if you tied off the lymphatics you could protect the animal from the effects of poisons provided that the poisons were not small enough to be absorbed by the blood vessels. The lymphatics can play quite a big part, and I sometimes wonder if the crystals of crystal suspensions do not get taken up by the lymphatics and distributed about the body without waiting to be dissolved.

One method which is used with some drugs is to add a vaso-constrictor. In most of the cases we have been considering a vaso-constrictor would not act long enough. You can prolong the action of a local anæsthetic for quite a long time by adding a vaso-constrictor, but you can get much more prolonged effects by using insoluble local anæsthetics such as benzocaine. I wonder if it may not be a good thing to add benzocaine to some of those injections which are going to be absorbed slowly, in order to avoid painful effects?

Substances can be given in solution with the idea that they will be precipitated in the body. Steroids can be given in propylene glycol which is distributed widely in the tissues

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This is one factor which affects the duration of action of drugs. With some other drugs you do not inject the really active principle itself, but the body forms the active principle after the drug has been injected. I do not know of any examples of that in the steroid field, but, of course, neoarsphenamine is a pentavalent arsenical which does not have any therapeutic effect itself, but has to wait to be converted in the body into a therapeutically active substance. Phenacetin is another drug which is not active by itself but which is probably converted in the body into para-amino-phenol which is liberated slowly and gives the therapeutic action.

Another method of affecting the duration of action of drugs is to modify their rate of destruction. Barbiturates have a much briefer action when they have a long tail attached, which makes them much more unstable so that they are more readily destroyed in the body. I don't think that we know enough about the metabolism of steroids to be able to alter their rate of destruction in the body. Then there are some drugs which are very slowly excreted because they are firmly bound to the tissues; digitoxin, for example, has a very prolonged action, because once it has got into the heart it cannot get out again, whereas allied substances with a similar action are less firmly bound to the tissues, and have a briefer action.

Another method of prolonging destruction is to delay excretion; para-amino hippuric acid, and now caronamide, has been used to delay the excretion of penicillin.

These are some of the techniques which have been used in other fields; perhaps some of them will be applied some day to steroids.

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